DIET COMPOSITION AND FATE OF CONTAMINANTS IN SUBSISTENCE HARVESTED NORTHERN SEA OTTERS (*ENHYDRA LUTRIS KENYONI*) FROM ICY STRAIT, ALASKA

By

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Abstract

Northern sea otters (*Enhydra lutris kenyoni*) in Southeast Alaska have experienced a significant population increase since their successful reintroduction to the area after previous near extirpation owing to historic fur trading. The purpose of this study was to examine sea otter diet and metals contamination in an area of Southeast Alaska with the most robust increases in sea otter numbers, Glacier Bay/Icy Strait, with the intent of gathering baseline data for a healthy population of sea otters and as a reflection of the local coastal environmental health of the area. This research was a collaborative effort with Alaska Native subsistence hunters and the Alaska Department of Environmental Conservation. In Chapter 1, sea otter stomachs (n=25) were obtained in April 2015 and April 2016 from Alaska Native subsistence hunters in Icy Strait, Alaska. There were no differences in sea otter diet between years. Bivalves dominated the sea otter diet. Northern horsemussels (*Modiolus modiolus*) made up the greatest proportion of the diet (0.46 ± 0.48). Fat gaper clams (*Tresus capax*) and northern horsemussels were found in the highest proportion of stomachs (0.64 and 0.60, respectively). There was not an apparent trend between sea otter age and the minimum number of total prey items, stomach contents mass, or mean frequency of occurrence of the top four prey species. Sea otters from this study are likely to be dietary generalists throughout their lives. In Chapter 2, brain, gonad, kidney, and liver tissues, as well as stomach contents were analyzed for arsenic, cadmium, copper, lead, total mercury, and selenium for the 2015-harvested sea otters that were also referenced in Chapter 1 (n=14). In general, arsenic and lead had the highest concentrations in stomach contents, cadmium and selenium were highest in the kidneys, and copper and total mercury were highest in the livers. While brains and gonads had the lowest metals concentrations of any tissue, the metal with the greatest concentration within the brain was copper, and within the gonads was selenium. Concentrations of arsenic, cadmium, total mercury, and lead demonstrated a relationship with sea otter length. In general, all the mean metals concentrations for these sea otters were below published effects threshold values for marine mammals. Only total mercury demonstrated biomagnification from the stomach contents (i.e., the prey) to all higher-level tissues. Selenium health benefit values were positive in all sea otter tissue types analyzed in the present study, indicating that concentrations of selenium had an

overall health benefit in protecting those tissues against mercury toxicity. Evaluating how contaminants concentrate and get distributed in tissues of top trophic levels provides an indication for potential exposure to humans and demonstrates how these keystone species act as indicators of local coastal ecosystem health. The results of studies on dietary exposure and metals contamination in top trophic level consumers such as sea otters can be used in monitoring the health of sea otter populations and the local environment that they inhabit.

Table of Contents

List of Figures

Page

List of Tables

Page

Dedication Page

I dedicate this body of work to my Dad, Richard Lynn Brown, who was always proud of me no matter what, and never missed the opportunity to tell me so. I miss him every day. His memory is what pushed me through to finishing this degree. I know he is celebrating this achievement with me in spirit.

Love you always, Dad.

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Statement of authorship: In chapter 1, I conducted most of the laboratory work, the entirety of the data analysis, and wrote the manuscript. My first co-author (Dr. Shannon Atkinson) aided our work in the laboratory and assisted extensively in the editing of the manuscript. My second co-author (Kierstin Keller) aided extensively with laboratory work and also collected the primary bivalve species from around Juneau and Douglas and compiled the photo reference guide for identification of stomach contents (Keller et al., 2017). My third co-author (Dr. Heidi Pearson) assisted with statistical analyses and significant manuscript editing. In chapter 2, I conducted half of the sample collection, a majority of the sample prep work, the entirety of the data analysis, and wrote the manuscript. Dr. Shannon Atkinson conducted the other half of sample collection, assisted extensively in the editing of the manuscript, and aided the sample prep work. Dr. Christoff Furin oversaw the laboratory analyses, and assisted significantly in the data and statistical analyses. Dr. Franz Mueter assisted extensively in the statistical analyses. Dr. Robert Gerlach aided in project and analysis design. The Alaska Environmental Health Laboratory in Anchorage, Alaska conducted all the metals analyses.

General Introduction

Sea otters (*Enhydra lutris*) are widely recognized as a keystone species due to their significant influence in shaping the community structure of their ecosystem (Estes and Palmisano, 1974; Garshelis et al., 1986; Estes, 1990; Jessup et al., 2004). They inhabit a broad coastal range around the North Pacific rim (Kenyon, 1981), and have been categorized into three subspecies according to their geographical distribution. The southern sea otter (*E. l. nereis*) is found in southern California, the Russian sea otter (*E. l. lutris*) is found along Alaska's Commander Islands and all the way to Asia, and the northern sea otter (*E. l. kenyoni*) can be found occupying northern California to the Aleutian Islands in Alaska (Estes, 1980; Mundy, 2005).

Overexploitation of sea otters during the 18th century Russian fur trade nearly eradicated sea otters from their original range within Alaska (Kenyon, 1969; Jameson et al., 1982). In 1911 the International Fur Seal Treaty afforded protection to sea otters (Elliott and Hay, 1911), but by that time less than 2,000 surviving individuals remained worldwide (Bodkin and Monson, 2002). Sea otters eventually began repopulating areas of their historic range, but continued to be absent from Southeast Alaska. Hence, translocation efforts were instated.

Successful reintroduction of 412 sea otters carried out by the Alaska Department of Fish and Game (ADF&G) from 1965 to 1972 has since resulted in a dramatic population increase to the Southeast Alaska region. Specifically, sea otters associated with the Glacier Bay National Park and Preserve demonstrated a 44% annual rate of increase between the time of reintroduction and population counts by aerial surveys conducted between 1999 and 2002 (Esslinger and Bodkin, 2009). While Glacier Bay's rate of increase is likely a combined result of increased birth rate and immigration, sea otters throughout all of Southeast Alaska experienced an annual growth rate of 18% per year between 1975 and 1987 (Estes, 1990), but slowed to 8.6% growth rate for the years between 2003 and 2011 (Tinker et al., 2019). In 2002, sea otter populations were estimated to be 1,266 in the lower part of Glacier Bay, and 3,104 in the combined area of Glacier Bay and northern Southeast Alaska (Esslinger and Bodkin, 2009). Between 2002 and 2012, sea otters in Glacier Bay demonstrated an annual growth rate of 20.6%, leading to a

population count of 7,955 (Tinker et al., 2019). The United States Fish and Wildlife Service (USFWS) estimates the overall population of Southeast Alaska to be at 25,712 sea otters, of which 8,508 reside in Glacier Bay (USFWS, 2014). This estimate is more than double the population number cited in the previous stock assessment for the Southeast Alaska region of sea otters (10,563; USFWS, 2008).

Sea otters in Alaska primarily prey upon clams (*Bivalvia sp.*), mussels (*Mytilidae sp.*), crabs (*Brachyura sp.*), sea urchins (*Echinoidea sp.*), and sea cucumbers (*Holothuroidea sp.*) (Garshelis et al., 1986; Kvitek et al., 1993; Wolt et al., 2012; Larson et al., 2013). During the time of sea otters' extirpation, their prey species were released from predation pressure and flourished. Lucrative dive fisheries for clam, sea urchin, sea cucumber, and crab species became established in Southeast Alaska (USFWS, 1993; Larson et al., 2013). Because sea otters feed on a variety of species important to commercial, subsistence, and recreational fisheries, their recolonization and population increase has since resulted in direct competition with humans (Estes, 1990; USFWS, 1993; Larson et al., 2013; Hoyt, 2015).

It is estimated that in all Southeast Alaska, impacts from sea otters have affected 22% of the sea urchin, 18% of the sea cucumber, and 66% of the geoduck (*Panopea generosa*) fisheries. An additional 7% of the sea urchin and 4% of the sea cucumber fisheries have been shut down (Kenyon, 1981; Carswell, 2015). Sea otter predation was observed on 59% of transects where sea otters overlapped geoduck clam beds in Southeast Alaska between 1997 and 2013 (Hoyt, 2015). It took only five years for sea otters' presence to reduce the Dungeness crab (*Cancer magister*) fishery, and less than 3 years to reduce the red sea urchin (*Mesocentrotus franciscanus*) fishery, down to non-commercial levels (Hoyt, 2015). Larson et al. (2013) demonstrated a decrease in Southeast Alaska sea cucumber density by 100% after sea otters had colonized in 1994, by 80.1% after sea otters had recolonized in 2003, and by 25.8% after recolonization in 2010 (as compared to control areas which only experienced 19.6%, 19.1% and 15.4% decline, respectively).

Reduced catches of sea urchin, crab, geoduck, and sea cucumber were assessed at an intrinsic loss of 23.8 million dollars to the Alaska economy from sea otter predation (McDowell Group, Inc., 2011). Although this loss is both direct and indirect, the assessment states that shellfish and dive fisheries may

not be possible in the future if no measures are put in place to regulate sea otter numbers (McDowell Group, Inc., 2011). Commercial fishing combined with decreased resources (presumably owing to sea otter predation), has resulted in at least 18 dive fisheries district closures in Southeast Alaska since 1993 (Hebert, 2014). Conflicts continue to escalate especially because there is a lack of management in place to prevent sea otters from consuming resources upon which Alaska communities depend. Management has been discussed for Alaska, along with Russia and California, but no substantial measures have been taken (Garshelis et al., 1986; USFWS, 1993).

While the expansion of sea otters has caused declines in commercial fisheries resources, the presence of sea otters also supports diversity and resilience of their local coastal marine ecosystem. Secondary productivity is promoted through sea otters' consumption of herbivorous invertebrates (sea urchins, in particular), resulting in the revival of kelp (Order Laminariales) canopies (Estes and Palmisano, 1974; Shelton et al., 2018). Kelp forests are highly productive ecosystems supporting a variety of fish, invertebrate, and understory algal species that receive shelter and nutrients from the kelp (Estes and Palmisano, 1974; Jessup et al., 2004; Mundy, 2005). Without sea otters, kelp forests get reduced to urchin barrens (Jessup et al., 2004; Shelton et al., 2018). Kelp forests help also mitigate carbon dioxide in the atmosphere (Wilmers et al., 2012). The increase in ecosystem carbon owing to sea otters' indirect effect of increasing kelp forest abundance has been estimated at 205-408 million dollars (based on the 2012 European Carbon Exchange, converted to US dollars; Wilmers et al., 2012). Wilmers et al. (2012) went on to propose an interesting management solution to the re-introduction of sea otters and their direct effect in reducing fisheries: by selling the ecosystem carbon sequestered by the trophic cascade between sea otters and kelp forests. Although, Shelton et al. (2018) found that changes in kelp canopy abundance may not simply be based on sea otter presence alone, but that the effect sea otters have on kelp canopies may decrease over time or that kelp canopies may instead be more heavily influenced by environmental factors such as the impacts from El Niño and El Niña events in the Northeast Pacific, or changes in sea surface temperature, nutrient availability, or upwelling. Despite their perceived negative impacts on

fisheries, the presence of sea otters correspondingly results in positive ecological effects on the environments in which they inhabit.

Unlike other marine mammals, sea otters are small in body size and lack a blubber layer for insulation or energy stores. They must rely on their dense fur to stay warm (Kenyon, 1981). A single sea otter consumes roughly a quarter of its overall body weight per day to keep up with its high metabolism (Monson et al., 2000; Estes et al., 2003; Gilkinson et al., 2011). Sea otters forage in a wide range of substrates that are generally characterized as either (1) soft- and mixed-sediment or (2) rocky habitat (Kvitek et al., 1993). Sea otters living in mixed- and soft-sediment habitats have markedly different diets than sea otters from rocky habitats. Sea otters living in rocky substrates more typically prey upon sea urchins, large crustaceans, abalone (*Haliotidae sp.*), and fish (USFWS, 1993; Wolt et al., 2012; Newsome et al., 2015). Sea otters from areas of mixed- or soft-sediment benthos tend to have diets that are dominated by infaunal bivalves (Garshelis et al., 1986; Riedman and Estes, 1990; Kvitek et al., 1993). For Southeast Alaska sea otters, Washington butterclams (*Saxidomus gigantea*) have been shown to dominate sea otter diets (Kvitek and Oliver, 1992; Weitzman, 2013) representing an average 75% of the diet (Kvitek et al., 1993). Sea otters occupying soft-sediment habitats have been described as dietary generalists (Kvitek and Oliver, 1992; Wolt et al., 2012). However, it may be that there is simply a lack of dietary specialization in soft-sediment communities due to less overall prey diversity. Sea otters have been shown to have individual variation in their diet (Estes et al., 2003; Newsome et al., 2015), and prey specialization occurs most often in kelp forested communities where there are limited prey resources and lower inter-specific competition (Estes et al., 2003).

In areas that have long-established sea otter populations, mussels may become a primary prey source when other species are depleted. In areas with low-density sea otter populations, large quantities of sea urchins are preyed upon (USFWS, 1993). Although sea urchins are consumed in large numbers in rocky habitats, they provide a short-term prey source, whereas bivalves offer a longer-lasting nutritional supply (Kvitek and Oliver, 1992; Kvitek et al., 1993; Newsome et al., 2015). Laidre and Jameson (2006) noted that sea urchin predation was primarily observed from sea otters occupying new habitat (defined as

<4 years occupancy). As sea otters expanded their range and began depleting this preferred prey source, prey preference shifted to a more bivalve-dominated diet.

It is clear that sea otter foraging ecology has been well studied, primarily through direct foraging observations (Garshelis et al., 1986; Kvitek et al., 1993; Watt et al., 2000; Estes et al., 2003; Laidre and Jameson, 2006; Doroff et al., 2012; Wolt et al., 2012), stable isotope analysis (Newsome et al., 2009, 2015), or via scat analysis (Watt et al., 2000; Maldini et al., 2010; Parry et al., 2011; Doroff et al., 2012). However, diet composition studies via stomach contents analysis has not been examined in healthy sea otters (i.e., recently harvested instead of beached carcasses) since the 1960s, which was prior to their federal protection as a species, and was only accomplished for sea otters in the Aleutian Archipelago of Alaska (Kenyon, 1969). Stomach contents contain the actual organisms being eaten by an animal. Although providing only a snapshot in time of the diet, stomach contents analysis has the advantage of providing a high degree of taxonomic precision of prey species, prior to substantial digestion (Chipps and Garvey, 2007).

Compared to other marine mammals, sea otters live within a relatively small home range and do not migrate. They collect all of their prey resources from one general area and are therefore an excellent species for examining contaminants affecting a particular geographic area (Bacon et al., 1999; Comerci et al., 2001; Jessup et al., 2004; Kannan et al., 2008; Brancato et al., 2009). Sea otters target sessile prey which filter feed on sediments and detritus (or kelp, in the case of sea urchins) and can ingest and concentrate a variety of environmental contaminants (Bacon et al., 1999; Jessup et al., 2004; Carswell et al., 2015). In fact, diet is the primary pathway of exposure to environmental contaminants. As humans consume some of the same seafood items which are also heavily consumed by sea otters, contaminant levels in sea otter tissues offer a glimpse at what humans may be exposed to, albeit on a smaller scale considering that humans are eating a small fraction of the same foods that sea otters consume on a daily basis. There have been limited reports of Alaska Natives consuming sea otter meat, though they were previously led to believe they would contract leprosy by eating the meat; likely a myth spread by the Russians in their efforts to obtain more sea otter pelts (M. Gho, personal communication, 2018).

Sea otters are now protected under the Marine Mammal Protection Act of 1972 (16 U.S.C. § 1361 *et seq.* MMC, 2007), and under the Endangered Species Act for northern sea otters in Southwest Alaska (70 F.R. 46366-46386, 2005) and for southern sea otters in California (42 F.R. 2965-2968, 1977). Northern sea otters are the primary focus of the present study. This population of sea otters in U.S. waters is managed by the USFWS. Only Alaska Natives are allowed to hunt sea otters as part of their tradition and for subsistence purposes (50 C.F.R. § 18.23, 2000), with the exception of strictly prohibited hunting within Glacier Bay National Park and Preserve (USWFS, 1993; Esslinger and Bodkin, 2009).

Given the strong conservation measures in place for the species, much of sea otter research is logistically difficult and many studies must utilize stranded sea otter carcasses to obtain data (Morejohn et al., 1975; Kubota et al., 2001; Kannan et al., 2006, 2008; Brancato et al., 2009). While this is an adequate and advantageous way to opportunistically collect scientific data, it is challenging to analyze such samples for stomach contents analysis or for contaminant concentrations due to unknown prior conditions of the animal. The cause of death may well be attributed to abnormally high contaminant concentrations for the animal, starvation, or an illness that suppressed or eliminated appetite. Contaminant concentrations measured in diseased, starving, or just generally unhealthy sea otters are unlikely to accurately represent the true body burden of healthy animals from a robust population. In addition, animals that washed ashore from starvation would not have any stomach contents left to analyze. Evaluating how contaminants bioaccumulate and are distributed in tissues of sea otters for which we know the exact prey they consumed can shed light on potential exposures to humans. If humans are harvesting sea otters (via subsistence) and/or shellfish (via subsistence or recreationally) for consumption, they will undoubtedly bioaccumulate contaminants from those prey items, some of which can biomagnify in top level consumers (which includes humans and sea otters).

Selected metals arsenic, cadmium, copper, lead, total mercury, and selenium were the focus of the present study. Although primary contaminant exposure comes from food, many marine ecosystems are significantly influenced by metals entering the environment through various industrial, agricultural, pharmaceutical, and atmospheric sources with mining and smelting operations as primary point source

areas for heavy metals (USEPA, 2000). Elevated concentrations of heavy metals have been reported on a global scale in many marine animals such as sea otters, harbor seals (*Phoca vitulina*), northern fur seals (*Callorhinus ursinus*), grey seals (*Halichoerus grypus*), ringed seals (*Pusa hispida*), Steller sea lions (*Eumetopias jubatus*), beluga whales (*Delphinapterus leucas*), narwhals (*Monodon monoceros*), manatees (*Trichechus sp.*), polar bears (*Ursus maritimus*), and Pacific walruses (*Odobenus rosmarus divergens*) (Goldblatt and Anthony, 1983; Miles et al., 1992; Warburton and Seagars, 1993; Giger and Trust, 1997; AMAP, 1998; Eisler, 1998; Wagemann et al., 1998; Comerci et al., 2001; Kubota et al., 2001; Kannan et al., 2006; Habran et al., 2013; Rea et al., 2013; Noël et al., 2016). Heavy metals have been widely studied in sea otters from Russia, California, Washington, and Southcentral and Southwest Alaska (Comerci et al., 2001; Kubota et al., 2001; Kannan et al., 2006, 2008). However, relatively few studies have examined contaminants in the Southeast Alaska sea otter population (Comerci et al., 2001). Furthermore, previously conducted studies appear to have only ever evaluated kidneys and livers, and in one study, whole blood. Additionally, a majority of sea otters used in such studies were beach-cast carcasses, and therefore not representative of metal concentrations in the healthy population.

While some metals are considered essential for life (copper and selenium), they can lead to adverse effects when in surplus or deficiency. Selenium is highly toxic at excessive doses on its own but can reduce the negative effects of other heavy metals including arsenic, cadmium, lead, copper, and mercury. All of the selected metals for the present study are naturally occurring elements, however the United States Environmental Protection Agency (USEPA) ranks them as a priority in public health significance due to their high degree of toxicity, classification as carcinogens, and potential for inducing multiple organ damage even at low levels of exposure (USEPA, 2000). Determining where contaminants get distributed in the body is vital to interpreting their impacts on the physiology of an organism. Yet, there have been apparently no studies conducted on metals concentrations in the brain and gonad tissues of sea otters in any region, or on the distribution of contaminants to various sea otter tissues, particularly for the Southeast Alaska population of sea otters.

Through an ongoing collaboration with local Alaska Native subsistence hunters, I was granted access to the samples from sea otters collected near Glacier Bay, in Icy Strait, outside of Gustavus, Alaska. Glacier Bay waters are typically considered "pristine" (Bacon et al., 1999; Carswell et al., 2015), and it is particularly important to determine levels of contaminants in a pristine environment as a means of comparison for data gathered from less-pristine waters. Gathering baseline contaminant data while also knowing what the sea otters in this area are eating can help determine whether these waters truly are pristine or not. Furthermore, the Southeast Alaska Network of the National Park Service is currently in the process of implementing a protocol for sea otter monitoring in the Glacier Bay National Park and Preserve, as part of their Vital Signs Monitoring Program. This program was designed to "monitor the status and trend of key natural resource elements so that park managers can effectively preserve them" (Moynahan and Johnson, 2008). It is obvious that sea otter research is needed, particularly for Southeast Alaska where their population is increasing at such unprecedented rates, causing concern regarding their effects to commercial fisheries, and indicating the necessity for strategic management of both sea otters and commercially important fishery resources (Larson et al., 2013; Carswell et al., 2015; Hoyt, 2015; Tinker et al., 2019).

It is exceptionally advantageous to work with Alaska Native subsistence hunters when collecting scientific samples. The samples for this study were opportunistically collected from recently killed, but otherwise seemingly healthy sea otters. As previously mentioned, much of sea otter data come from beached carcasses and having fresh samples provides a more accurate measure of both contaminants and prey items still inside the stomachs. Not only is it helpful for researchers, but Alaska Natives can benefit from our assessment of the health and status of the marine mammal populations they are harvesting. As sea otters are keystone species capable of completely restructuring their local ecosystem, and also live and eat locally, their health is highly indicative of their surrounding ecosystem health. Alaska Natives are likely to be particularly invested in the good health of ecosystems in which they subsistence hunt so that they may continue their traditions for many years to come.

The overall goal of this study was to examine diet via stomach contents analysis and evaluate metals contamination in a healthy and expanding population of sea otters to obtain baseline data for the Glacier Bay/Icy Strait coastal marine ecosystem of Southeast Alaska. Based on previous foraging observations of sea otters in Southeast Alaska (Kvitek and Oliver, 1992; Kvitek et al., 1993) and specifically inside Glacier Bay National Park and Preserve (Weitzman, 2013), it was expected that the contents of sea otter stomachs in this study would primarily contain bivalves. Furthermore, as the samples were collected outside of Gustavus, Alaska near Glacier Bay and Glacier Bay waters are typically considered "pristine" (Bacon et al., 1999; Carswell et al., 2015), it was expected that metals concentrations would all be low compared to sea otters residing in other regions. The samples for the present study were collected from recently killed, but otherwise seemingly healthy sea otters that were part of Alaska Native subsistence hunts.

In Chapter 1, whole stomachs collected from 32 northern sea otters in Icy Strait, Alaska were dissected and the prey items inside each stomach were categorized by species with the overall goal of determining diet composition of what was actually being eaten by this group of apparently healthy sea otters. The specific objectives were to (1) identify prey at the species level; (2) determine frequency of occurrence of prey species found in sea otter stomachs; (3) determine the proportion of sea otter stomachs containing each prey item; and (4) examine how sea otter age relates to (i) minimum number of total prey items, (ii) stomach contents mass, and (iii) mean frequency of occurrence of the top four prey species.

In Chapter 2, brain, gonad, kidney, liver, and whole stomachs were collected from 14 northern sea otters in Icy Strait, Alaska and analyzed for concentrations of arsenic, cadmium, copper, lead, total mercury, and selenium. The specific objectives of this study were to (1) determine concentrations of selected metals in different sea otter tissues (brain, gonad, kidney, and liver); (2) determine whether selected metals biomagnify from stomach contents (i.e., the prey) to other sea otter tissues; (3) determine whether molar concentrations of selenium and mercury indicates an overall health benefit or risk to sea otters; and (4) determine if selected metals concentrations in sea otter tissues vary with sea otter size.

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Chapter 1:

Diet of northern sea otters (*Enhydra lutris kenyoni*) from Icy Strait, Alaska, based on stomach contents analysis[1]

1.1. Abstract

Sea otter (*Enhydra lutris*) diet through stomach contents analysis has not been examined in healthy sea otters since the 1960s, prior to their federal protection as a species, and was only accomplished for sea otters in the Aleutian Archipelago. Samples for the present study were collected from recently harvested, but otherwise seemingly healthy, sea otters collected during Alaska Native subsistence hunts in Southeast Alaska. The specific objectives of this study were to (1) identify prey at the species level; (2) determine frequency of occurrence of prey species found in sea otter stomachs; (3) determine the proportion of stomachs containing each prey item; and (4) examine how sea otter age relates to (i) minimum number of total prey items, (ii) stomach contents mass, and (iii) mean frequency of occurrence of the top four prey species. Northern horsemussels (*Modiolus modiolus*) made up the greatest proportion of the diet (0.46 ± 0.48). Fat gaper clams (*Tresus capax*) and northern horsemussels were found in the highest proportion of stomachs (0.64 and 0.60, respectively). There were no apparent trends observed between the age of a sea otter and the variables examined. The predominance of bivalves likely reflects the soft-sediment community where the sea otters of the present study were harvested.

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1.2. Introduction

Sea otters (*Enhydra lutris*) are widely recognized as a keystone species due to their significant influence in shaping the community structure of their ecosystem (Estes and Palmisano, 1974; Garshelis et al., 1986; Estes, 1990; Jessup et al., 2004). Overexploitation of sea otters during the 18th century Russian fur trade eradicated sea otters from their original range within Southeast Alaska (Kenyon, 1969; Jameson et al., 1982). In the absence of sea otters, lucrative dive fisheries for clam (*Bivalvia sp.*), sea urchin (*Echinoidea sp.*), sea cucumber (*Holothuroidea sp.*), and crab (*Brachyura sp.*) species were established in Southeast Alaska (USFWS, 1993; Larson et al., 2013).

Successful reintroduction of 412 sea otters carried out by the Alaska Department of Fish and Game from 1965 to 1972 (Jameson et al., 1982) has since resulted in a dramatic population increase to the Southeast Alaska region. Specifically, sea otters associated with the Glacier Bay National Park and Preserve have demonstrated a 44% annual rate of increase since population counts by aerial surveys began in 1999 (Esslinger and Bodkin, 2009). While Glacier Bay National Park and Preserve's rate of increase is likely a combined result of increased birth rate and immigration, sea otters throughout all of Southeast Alaska experienced an annual growth rate of 18% per year between 1975 and 1987 (Estes, 1990). However, between the years 2003 and 2011, the annual growth rate of Southeast Alaska sea otters slowed to 8.6% (Tinker et al., 2019). In 2002, sea otter populations were estimated to be 1,266 in the lower part of Glacier Bay, and 3,104 in the combined area of Glacier Bay and northern Southeast Alaska (Esslinger and Bodkin, 2009). Between 2002 and 2012, sea otters in Glacier Bay demonstrated an annual growth rate of 20.6%, leading to a population count of 7,955 (Tinker et al., 2019). The United States Fish and Wildlife Service (USFWS) stock assessment reports the overall population of Southeast Alaska to be at 25,712 sea otters, of which 8,508 reside in Glacier Bay (USFWS, 2014). This estimate is more than double the population number cited in the previous stock assessment for the Southeast Alaska region of sea otters (10,563; USFWS, 2008).

Because sea otters feed on a variety of species important to commercial, subsistence, and recreational fisheries, their population increase has resulted in direct competition with humans (Garshelis

et al., 1986; Estes, 1990; USFWS, 1993; Larson et al., 2013; Carswell et al., 2015). It is estimated that in all of Southeast Alaska, impacts from sea otters have affected 22% of the sea urchin, 18% of the sea cucumber, and 66% of the geoduck (*Panopea generosa*) fisheries. An additional 7% of the sea urchin and 4% of the sea cucumber fisheries have been shut down (Kenyon, 1981; Carswell et al., 2015). Sea otter predation was observed on 59% of transects where sea otters overlapped geoduck clam beds in Southeast Alaska between 1997 and 2013 (Hoyt, 2015). It took only five years for sea otters' presence to reduce the Dungeness crab (*Cancer magister*) fishery, and less than 3 years to reduce the red sea urchin (*Mesocentrotus franciscanus*) fishery, down to non-commercial levels (Hoyt, 2015). Since 1994, sea cucumber densities were reduced up to 100% in Southeast Alaska areas recolonized by sea otters (Larson et al., 2013).

Reduced catches of sea urchin, crab, geoduck, and sea cucumber were assessed at an intrinsic loss of 23.8 million dollars to the Alaska economy from sea otter predation (McDowell Group, Inc., 2011). Although this loss is both direct and indirect, the assessment states that shellfish and dive fisheries may not be possible in the future if no measures are put in place to regulate sea otter numbers (McDowell Group, Inc., 2011). Conflicts continue to escalate especially because there is a lack of management in place to prevent sea otters from consuming resources upon which Alaska communities depend. Management has been discussed for Alaska, along with the Soviet Union (now the Russian Federation) and California, but no substantial measures have been taken (Garshelis et al., 1986; USFWS, 1993).

On average, sea otters consume nearly 25% of their overall body weight per day (Estes et al., 2003). Based on previous studies, sea otters in Alaska primarily feed on clams, mussels (*Mytilidae sp.*), crabs (Dungeness, in particular), sea urchins, and sea cucumbers, with clams comprising over 70% of the diet in soft-bottom habitat (Garshelis et al., 1986; Kvitek et al., 1993; Wolt et al., 2012; Larson et al., 2013). Sea otter diet composition has been well studied, primarily through direct foraging observations (Garshelis et al., 1986; Kvitek et al., 1993; Watt et al., 2000; Estes et al., 2003; Laidre and Jameson, 2006; Doroff et al., 2012; Wolt et al., 2012), stable isotope analysis (Newsome et al., 2009, 2010, 2015), or via scat analysis (Watt et al., 2000; Maldini et al., 2010; Parry et al., 2011; Doroff et al., 2012). However, diet

composition by stomach contents analysis has not been examined in healthy sea otters (i.e., recently harvested as opposed to beached carcasses) since the 1960s, prior to their federal protection as a species, and was only ever published for sea otters in the Aleutian Archipelago of Alaska (Kenyon, 1969).

Sea otters living in mixed and soft sediment habitats have markedly different diets than sea otters from rocky habitats. Burrowing bivalves are the main source of prey for sea otters foraging in soft-sediments, such as those in Prince William Sound where sea otter diet is composed of 34-100% bivalves (Wolt et al., 2012). In Simpson Bay and Kodiak, the main invertebrates consumed are also bivalves, making up 70-80% of sea otter diets (Wolt et al., 2012). For Southeast Alaska sea otters, Washington butterclams (*Saxidomus gigantea*) comprise an average of 75% of the diet (Kvitek et al., 1993). In areas that have long-established populations, mussels may become a primary prey source when other species are depleted. Bivalves offer a longer-lasting nutritional supply than other prey sources (Kvitek and Oliver, 1992; Kvitek et al., 1993; Newsome et al., 2015).

Sea otters are protected under the Marine Mammal Protection Act of 1972 (16 U.S.C. § 1361 *et seq.* MMC, 2007), and under the Endangered Species Act for northern sea otters (*E. l. kenyoni*) in Southwest Alaska (70 F.R. 46366-46386, 2005) and for southern sea otters (*E. l. nereis*) in California (42 F.R. 2965-2968, 1977). Northern sea otters, the primary focus of the present study, occupy the middle-range of the North Pacific rim from northern California to the Aleutian Islands in Alaska and in the Commander Islands at the westernmost end of the Aleutian Archipelago. This population of sea otters in U.S. waters is managed by the USFWS. Only Alaska Natives are allowed to hunt sea otters as part of their tradition and for subsistence purposes (50 C.F.R. § 18.23, 2000), with the exception of strictly prohibited hunting within Glacier Bay National Park and Preserve (USFWS, 1993; Esslinger and Bodkin, 2009). Given the strong conservation measures in place for the species, research on sea otter stomach contents is logistically difficult and no studies have been published on this topic since Kenyon (1969).

Collaborating with Alaska Native subsistence hunters to obtain whole stomachs from freshly harvested sea otters, the goal of the present study was to use stomach contents analysis to determine diet composition of a healthy and expanding population of sea otters in Southeast Alaska. Specific objectives

were to 1) identify prey items to the species level; (2) determine frequency of occurrence of prey species found in sea otter stomachs; (3) determine the proportion of sea otter stomachs containing each prey item; and (4) examine how sea otter age relates to (i) minimum number of total prey items, (ii) stomach contents mass, and (iii) mean frequency of occurrence of the top four prey species. Based on previous foraging observations of sea otters in Southeast Alaska (Kvitek and Oliver, 1992; Kvitek et al., 1993) and specifically inside Glacier Bay National Park and Preserve (Weitzman, 2013), it was expected that the contents of sea otter stomachs in this study would primarily contain bivalves.

1.3. Methods

1.3.1. Sample Collection

Thirty-two sea otters (27 males, 5 females) were harvested by Alaska Native subsistence hunters near Glacier Bay, in Icy Strait, outside of Gustavus, Alaska (Figure 1.1). A premolar or incisor tooth was removed from the skull of each sea otter by the hunter in the field for age analysis. Whole stomachs were taken from each of the freshly harvested sea otters in April 2015 (n=14) and April 2016 (n=18). Zip ties were used to tie off both ends of the stomachs before cutting them out of the body. All stomach samples were collected in the field, stored chilled with ice packs, and brought back to the laboratory at the University of Alaska Fairbanks (UAF) Juneau Center of the College of Fisheries and Ocean Sciences (JC-CFOS) for further processing. Prior to analysis, a representative sample of contents from 13 of the 14 stomachs collected in 2015 (one stomach was empty) was hand-picked for contaminant analysis for use in a companion study (Brown et al.[2]). The rest of the 2015 stomach contents and 2016 whole stomachs were retained at the UAF JC-CFOS for species identification. A photo reference guide allowing identification of bivalves by their siphons and feet (as these were the most notably identifiable features found in the stomachs) was developed from a collection of primary bivalve species from around the Juneau, Alaska region (Keller et al., 2017). This photo reference guide was then used to identify the bivalve prey items

[2] Brown, K.L., Atkinson, S., Furin, C.G., Mueter, F.J., Gerlach, R. (2020). Metals in the stomach contents and brain, gonad, kidney, and liver tissues of subsistence-harvested northern sea otters (*Enhydra lutris kenyoni*) from Icy Strait, Alaska. Manuscript in preparation for Marine Pollution Bulletin.

inside each sea otter stomach to the species level. The only prey species that was not a bivalve species was the sea star (*Asteroidea sp.)*, which was identified subjectively based on the researchers' knowledge of sea star anatomy and the tube feet that were observed on the sea star arms found in four of the sea otter stomachs.

1.3.2. Stomach Contents Analysis

Each of the stomachs was weighed to the nearest 0.1 g. Each stomach was individually cut open and the contents were poured out and drained using a fine mesh net. A volume measurement to the nearest 0.1 mL was obtained for the stomach fluid, and the stomach linings were weighed separately to the nearest 0.1 g. All measurements were obtained using a Mettler PE 2000 scale. Empty stomachs (n=7) were not analyzed any further. Prey species were separated based on their identifiable features noted using a photo reference guide (Keller et al., 2017). A minimum number of individuals was counted for each prey species (i.e., the fewest possible number of each prey species identified within each stomach), either by the presence of a siphon and/or foot in the stomach contents. Bivalves have two siphons and one foot, although the siphons are typically conjoined, thus each siphon identified was counted as one individual (Figure 1.2). In cases where there were unequal numbers of siphons and feet, the higher number was used. In general, more siphons than feet could be identified. Prey items that could be accurately identified were combined by species and weighed to the nearest 0.1 g (wet weight). Individual prey items were not weighed. Sixteen stomachs had a portion of the contents that were too digested to identify, and thus weighed separately and recorded as 'unidentified biomass'. A group of siphons that was not represented in the photo reference guide (Keller et al., 2017), but clearly all belonged to the same bivalve species (i.e., all the siphons were identical to each other, but did not match any of the siphons of the bivalves in the photo reference guide), was recorded as 'unknown bivalve'.

A digestion rating and an identification (ID) confidence rating were assigned to each stomach. Digestion ratings were based on the percentage of unidentified biomass as compared to the overall biomass (Table 1.1), with the assumption that material which has been highly digested will not be as easily identified, and thus lead to a higher proportion of unidentifiable biomass. Identification confidence

ratings were based on the overall number of species identified in each stomach, with the assumption that a greater number of prey species in a single stomach leads to a higher level of uncertainty in accurate identification of each individual prey species (Table 1.2).

1.3.3. Age Analysis

Teeth were analyzed at Matson's Tooth Aging Laboratory (Manhattan, Montana) as was also done in Hutchinson et al. (2015) for the same population of sea otters. Growth layers in the cementum of the extracted premolar (standard tooth) or incisor (non-standard tooth) were used to obtain individual sea otter ages, based on one growth layer per year. One of the 12 teeth from the sea otters collected in 2015 and one of the 13 teeth from sea otters collected in 2016 were noted as having been broken with missing cementum; however, Matson's Laboratory was still able to accurately determine age for those individuals. One of the 12 teeth from the sea otters collected in 2015 was noted by Matson's Laboratory as being a non-standard tooth; however, again Matson's Laboratory determined that age analysis was not affected. Six total teeth (five from 2015 and one from 2016) were given an age range in addition to the age result provided. These six teeth were assigned a lower reliability index, indicating that there is histological evidence to support the age result provided and the correct age is expected to fall within the age range given. Age classes were assigned based on tooth age, and for the present study were defined as follows: pups/juveniles were <2 years old, subadults were between 2 and 4 years old, and adults were >4 years old.

1.3.4. Data Analysis

Statistical analyses were conducted using R and SPSS (IBM Corp., 2013; R Core Team, 2015) with an alpha level of $p<0.05$. Biomass of individual prey items was excluded from calculations for the present study, due to unknown ingestion and digestion times. Mann Whitney U t-tests were used to test whether frequency of occurrence differed between years; it did not and therefore data were combined for statistical analyses. For calculations, 'identifiable prey items' refers to all prey that were categorized by species, including the unknown bivalve species; this excludes any unidentifiable biomass.

Mean frequency of occurrence (mean FO) was defined as the proportion of the diet made up by a specific prey species. Frequency of occurrence (FO) was calculated for each stomach as:

$$FO = \left(\frac{n_i}{n}\right),$$

where n_i is the number of occurrences of prey item i, and n is the total number of occurrences of all identifiable prey items. Individual stomach FOs were then averaged for each prey species, to obtain the mean FO.

The proportion of stomachs with each prey item was calculated as:

$$Proportion\ of\ Stomachs\ with\ Each\ Prey\ Item = \left(\frac{s_i}{s}\right),$$

where s_i is the number of stomachs in which prey item i occurred and s is the total number of stomachs containing identifiable prey items in the sample.

Data were not normally distributed. Kendall rank correlation tests were used to examine the relationship between sea otter age and (i) minimum number of total prey items; (ii) stomach contents mass; and (iii) mean frequency of occurrence of the top four prey species. Statistical analyses could not be examined by sea otter sex because there were not enough females to provide unbiased results.

1.3.5. Power Analysis

Power analysis was conducted post-hoc using G*Power version 3.1.9.2 (Faul et al., 2009), as samples used for the present study were collected opportunistically by Alaska Native subsistence hunters. In post-hoc analysis, power $(1 - \beta)$ is computed as a function of significance level (α), population effect size (ES), and sample size (N). A two-tailed exact correlation bivariate normal model was run post-hoc, where $H_0: p - p_0 = 0$ and $H_1: p - p_0 \neq 0$. Correlation p for H_1 was set at 0.5 for large effect size (Cohen, 1992), α at 0.05, the present study's total sample size of N=25, and correlation p for H_0 was set at 0.

1.4. Results

In total, 25 stomachs were analyzed for stomach contents (Table 1.3) as seven of the 32 stomachs (21.9%) collected were empty. The sea otters that were used for stomach contents analysis ranged from 0 to 13 years of age; of those, 10 were assigned age classification as pups/juveniles, nine as subadults, and six as adults (Table 1.3). Preliminary gross observations indicated that the stomachs were primarily

comprised of bivalves (90-100%). Stomachs assigned a digestion rating of 1 (n=9), or 0% unidentified biomass compared to overall biomass, were always given an ID confidence rating of A (Tables 1.1 and 1.2). It is important to note that stomachs identified as containing only a single prey species did not have any "unidentifiable" parts. Stomachs assigned a digestion rating of 2 (n=4), 1-25% unidentified biomass, or 3 (n=8), 26-50% unidentified biomass, were assigned an ID confidence rating of either B or C. Stomachs assigned a digestion rating of 4 (n=2), 51-75% unidentified biomass, or 5 (n=2), 76-100% unidentified biomass, were assigned an ID confidence rating of C, with the exception of one stomach (No. 15-20) which received a digestion rating of 5 but an ID confidence level of A, indicating high confidence that everything in that particular stomach was of only one species, but that the material was also highly digested.

Six bivalve species and an unidentified species of sea star were observed in the sea otter stomach contents (Tables 1.4 and 1.5). A single stomach contained anywhere from zero prey items (empty stomachs) to a minimum of 47 prey items, with a single non-empty stomach containing between one and five differing species of prey (Table 1.5). The mean total minimum number of prey items in a single stomach was 17.8 (±13.7 SD, median = 13). The mean number of different prey species within a single stomach was 2.3 (±1.3 SD, median = 2). Forty percent (n=10) of stomachs contained only a single species of prey. Of the stomachs containing only a single prey species, northern horsemussel (*Modiolus modiolus*) was that singular species in 80% of stomachs. For the other two stomachs containing only a single prey species, one was made up of a single softshell clam individual (*Mya arenaria*) and the other a single fat gaper individual (*Tresus capax*) (Table 1.5).

Northern horsemussels made up the greatest proportion of prey species in sea otter stomachs (0.46 ± 0.48), followed by fat gaper clams (0.18 ± 0.27), the unknown bivalve species (0.11 ± 0.18), Washington butterclams (0.11 ± 0.22), softshell clams (0.07 ± 0.21), Arctic surfclams (*Mactromeris polynyma*) (0.06 ± 0.21), and sea stars (0.02 ± 0.05) (Table 1.4). Fat gaper clams and northern horsemussels were found in the greatest proportion of stomachs (0.64 and 0.60, respectively), followed by the unknown bivalve species (0.36), Washington butterclams (0.28), softshell clams and sea stars (both at

0.16), and Arctic surfclams (0.08) (Table 1.4). The top four prey species were northern horsemussels, the unknown bivalves, fat gaper clams, and Washington butterclams (Tables 1.4 and 1.5); these species made up 86% of the diet.

Power analysis indicated the present study sample size of n=25 had insufficient strength (power=0.75) to determine relationships between sea otter age and dietary variables. This was attributed to the opportunistic nature of sample collection through subsistence harvest. To achieve a large effect size (power=0.80), a minimum sample size of n=28 (Cohen, 1992) was required. While 32 total stomachs were collected (which would have provided sufficient power for statistical analyses), seven of the stomachs were empty and were excluded from analyses. Thus, results pertaining to age and diet are reported as trends only. There were no apparent trends observed between the age of a sea otter and the three variables tested: (i) the minimum number of total prey inside its stomach; (ii) the mass of that sea otter's stomach contents; and (iii) the mean frequency of occurrence of the top four prey species.

1.5. Discussion

Sea otters from areas of mixed- or soft-sediment benthos (such as that of the present study area) tend to have diets dominated by infaunal bivalves (Garshelis et al., 1986; Riedman and Estes, 1990; Kvitek et al., 1993; Maldini et al., 2010; Wolt et al., 2012). The bivalves found in the majority of sea otter stomachs and also the most prevalent species in the sea otter diet of the present study were fat gaper clams and northern horsemussels, whereas previous studies in Southeast Alaska have found the Washington butterclam to dominate sea otter diets (Kvitek and Oliver, 1992; Kvitek et al., 1993; Weitzman, 2013). However, sea otters have also been shown to have individual variation in their diet (Estes et al., 2003; Newsome et al., 2015) which could help explain the prevalence of certain bivalve species between studies.

There were no apparent trends observed between sea otter age and both the minimum number of total prey inside its stomach and the mass of its stomach contents. This suggests that sea otters may consume the same amount of prey items regardless of age and if so, that the stomachs of young and old sea otters may be physically capable of holding the same mass of prey. This also suggests that, regardless

of age, sea otters may need to maintain a critical mass of prey consumption in order to meet their high metabolic requirements. It is important to note that sea otters from the present study which had empty stomachs were in all age classes, ranging from 0 to 8 years. While not significant, a linear relationship between stomach contents mass and number of prey was observed during exploratory data analyses, indicating that a greater number of prey exists in the stomach contents when the mass of the stomach contents is large. This is important to note since it also indicates that a large mass of stomach contents is unlikely to be the result of a small number of very large prey items, but instead a large mass of stomach contents is made up of many prey items. As sea otter age did not show any apparent trend with the mean frequency of occurrence of the top four prey species found inside their stomachs, it may be concluded that sea otters in the present study are dietary generalists throughout their lives. This was also observed for sea otters living in the soft- and mixed-sediment habitat of Prince William Sound (Wolt et al., 2012). However, it is important to note that each stomach in the present study represents a snapshot in time of the sea otter diet and may not fully represent the entire diet of a given individual.

This study would not have been possible without the collaboration of Alaska Native hunters. Alaska Natives typically only utilize the pelt (and in some cases, meat) of the sea otters they hunt, and with proper permitting and protocols, researchers can have access to freshly harvested sea otters for a variety of research purposes, as was done in the present study. While the nature of sample collection precludes strict establishment of sample size, future work should aim for larger sample sizes to ensure sufficient statistical power. Further, although Alaska Natives primarily hunt sea otters in the spring and summer months (B. Benter, personal communication, 2016), whenever possible, it would be of interest to collect sea otter stomach contents from opposing seasons to test for seasonal changes in diet. Finally, sample collection over longer time periods would enable analysis of dietary shifts according to changes related to population dynamics. For example, it appears the present study area may be shifting from being primarily male-dominated to being more equally inhabited by males and females (M. Gho, personal communication, 2017), as is the typical pattern of recolonization (Laidre et al., 2009). Future research

should include a more robust comparison of sea otter diet between males and females to account for sex-specific differences in energetic needs that may influence prey selection (Esslinger et al., 2014).

Icy Strait is a soft-sediment benthos and therefore the predominance of bivalves found in sea otter diets from this area is likely a reflection of that soft substrate habitat from which the sea otters were harvested. A predominately bivalve diet has also been documented in other sea otter diet studies conducted in mixed- and soft-sediment habitats (Garshelis et al., 1986; Kvitek et al., 1993; Maldini et al., 2010; Wolt et al., 2012). The results of the present study update a previous study of sea otter diet based on stomach content analysis (Kenyon, 1969) and advance knowledge of sea otter diet in soft-sediment habitats in Alaska.

1.6. Acknowledgements

We thank the United States Fish and Wildlife Service, B. Benter, and M. Gho for their help in obtaining sea otter tissue samples, and Dr. R. Stone of National Marine Fisheries Service for assistance with the bivalve ID guide (NOAA technical memorandum NMFS-AFSC-341, 25p). We thank the Alaska Department of Environmental Conservation Contaminated Sites Program for providing the study site webmap (Figure 1.1) and for providing the senior author with a paid internship for the duration of this research. Many thanks to the Jessie O'Bryan McIntosh and Kathryn "K" E. and John Doyle scholarships, and the Marine Research, Training and Technology Fund at the University of Alaska Foundation. Sea otter samples for this study were obtained under United States Fish and Wildlife Service permit number MA81899A-0.

1.7. Literature Cited

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1.8. Figures

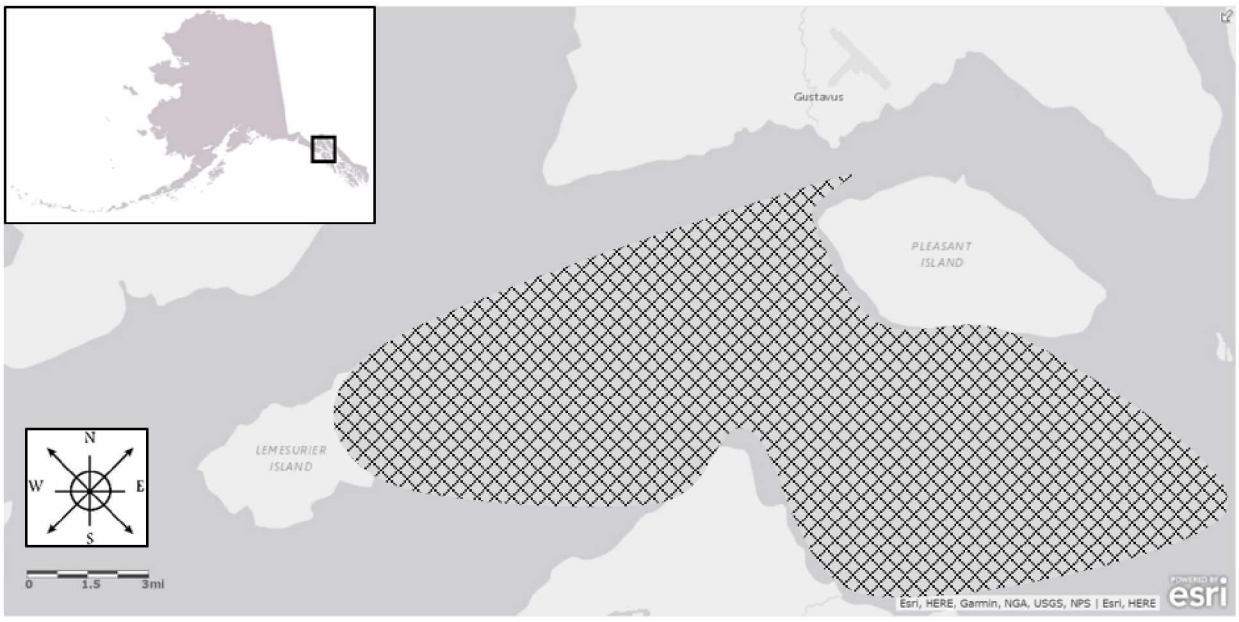

Figure 1.1. Study area. Map of the area in Icy Strait, Alaska where 32 northern sea otters (*Enhydra lutris kenyoni*) were harvested in April 2015 and April 2016. Whole stomachs were collected from each of the sea otters. The thatched section represents the approximate hunting area. The map was obtained and modified from the Alaska State Department of Environmental Conservation's Contaminated Sites Program public webmap, available online at: https://www.arcgis.com/home/webmap/viewer.html?webmap=315240bfbaf84aa0b8272ad1cef3cad3.

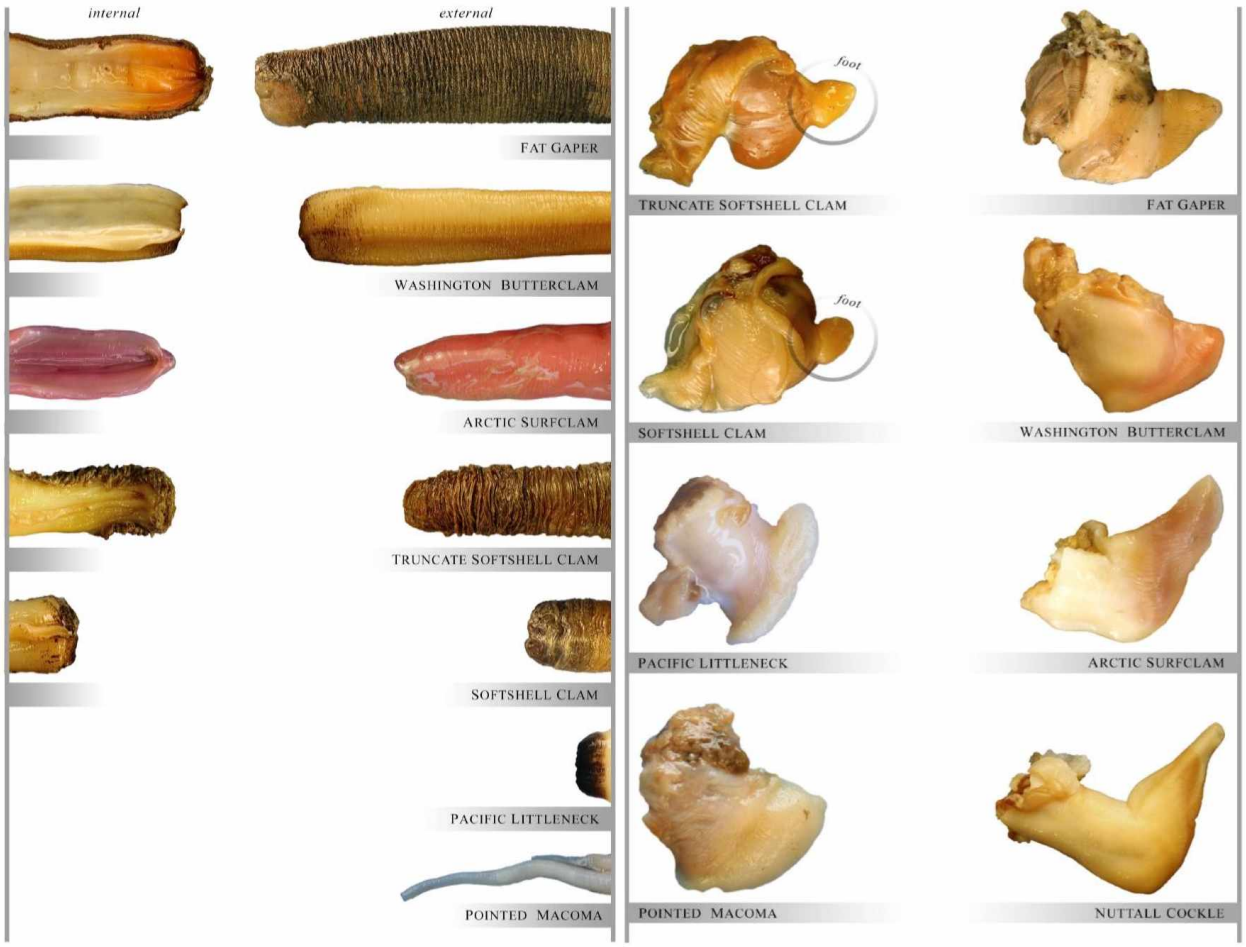

Figure 1.2. Bivalve comparison quick guides. Comparison of the siphons for seven bivalve species (left), and feet with attached sections of visceral mass for eight bivalve species (right) common to Southeast Alaska (used with permission from Keller et al., 2017). These charts were used for identifying bivalve species that were present inside the stomachs of northern sea otters (*Enhydra lutris kenyoni*) collected from Icy Strait, Alaska in April 2015 and April 2016 (n=25). The siphons of the Nuttall cockle (*Clinocardium nuttallii*) are not shown as they were too small and delicate to dissect and fell apart when the valves of this clam were opened (Keller et al., 2017).

1.9. Tables

Table 1.1. Digestion ratings. Digestion ratings were based on the percentage of unidentified biomass as compared to the overall biomass.

Digestion Rating	Percentage of Unidentified Biomass
1	0%
2	1-25%
3	26-50%
4	51-75%
5	76-100%

Table 1.2. ID confidence ratings. Identification (ID) confidence ratings were based on the overall number of prey species identified in each stomach, under the assumption that a greater number of prey species within a single stomach leads to a higher level of uncertainty in accurate identification.

ID Confidence Rating	Definition
A	Only one species in the entire stomach – 100% confident in identification.
B	Only two species in the entire stomach – confident in differentiating between the two species.
C	Three or more species in the entire stomach – not fully confident in differentiating between several species.

Table 1.3. Study animals. Northern sea otters (*Enhydra lutris kenyoni*) that were the subjects of the present study (n=25) with their sex, tooth age (calculated from an extracted tooth), assigned age class, the total mass of their stomach contents, the mass of their stomach linings, volume of their stomach fluid, and the assigned digestion and ID confidence ratings. See text for definitions. Sea otters with empty stomachs are excluded from the data presented here.

Sea Otter No.[a]	Sex	Tooth Age (years)	Age Class	Stomach Contents Mass (grams)	Stomach Linings Mass (grams)	Stomach Fluid Volume (mL)	Digestion Rating	ID Confidence Rating
15-09	F	10	Adult	343.6	174.5	125	3	C
15-12	M	2	Subadult	1287.4	191	290	5	C
15-13	M	4	Subadult	379.2	158.5	240	3	B
15-14	M	3	Subadult	865.3	165.7	260	4	C
15-15	M	2	Subadult	184.8	123.6	65	1	A
15-16	M	1	Pup/Juvenile	204.7	136.7	50	1	A
15-17	M	2	Subadult	758.7	186.2	198	1	A
15-18	M	4	Subadult	1105.4	213.3	375	2	B
15-19	M	7	Adult	551.8	193.6	240	1	A
15-20	M	5	Adult	231.5	209	140	5	A
15-21	F	10	Adult	1199.2	166	220	3	C
15-22	F	2	Subadult	32	136.2	5	1	A
16-03	M	0	Pup/Juvenile	432.5	168.4	120	3	B
16-05	M	1	Pup/Juvenile	257.5	114.3	150	1	A
16-08	M	1	Pup/Juvenile	86.3	107	44.5	1	A
16-09	M	1	Pup/Juvenile	133.4	162.4	5	3	C
16-10	M	13	Adult	421.9	182.5	219	3	B
16-11	M	1	Pup/Juvenile	331.8	182.6	15.5	4	C
16-12	M	2	Subadult	123.4	137.7	12	2	C
16-13	M	1	Pup/Juvenile	1108.3	210.9	255	3	C
16-14	M	1	Pup/Juvenile	1547.2	171.6	260	3	C
16-15	M	1	Pup/Juvenile	150.5	119.9	70	1	A
16-16	M	2	Subadult	284.5	203.7	44.5	2	C
16-17	M	1	Pup/Juvenile	418.1	140.8	53	2	B
16-18	F	4	Adult	946.8	244.8	450	1	A

Notes:

[a] Sea otter numbers start with the last two digits of the year that sea otter was harvested.

Table 1.4. Diet composition summary. Summary of prey items identified in northern sea otter (*Enhydra lutris kenyoni*) stomachs collected from Icy Strait, Alaska in April 2015 and April 2016 (n=25). Empty stomachs are excluded from these calculations. Mean frequencies of occurrence were not significantly different between years, and therefore data were combined to obtain the mean ± standard deviations presented here. See text for formulae and definitions.

Common Name	Scientific Name	Mean Frequency of Occurrence of Prey Item	Total Minimum Number of Prey Item for All Stomachs	Number of Stomachs with Prey Item	Proportion of Stomachs with Prey Item
Sea Star	*Asteroidea sp.*	0.02 ± 0.05	4	4	0.16
Arctic Surfclam	*Mactromeris polynyma*	0.06 ± 0.21	34	2	0.08
Fat Gaper	*Tresus capax*	0.18 ± 0.27	48	16	0.64
Northern Horsemussel	*Modiolus modiolus*	0.46 ± 0.48	233	15	0.60
Softshell Clam	*Mya arenaria*	0.07 ± 0.21	24	4	0.16
Washington Butterclam	*Saxidomus gigantea*	0.11 ± 0.22	47	7	0.28
Unknown Bivalve	NA	0.11 ± 0.18	56	9	0.36

Table 1.5. Minimum number of individuals. The minimum number of prey items counted inside individual northern sea otter (*Enhydra lutris kenyoni*) stomachs collected from Icy Strait, Alaska in April 2015 and April 2016 (n=25). Sea otters with empty stomachs are excluded from the data presented here. See text for definitions.

Sea Otter No.[a]	Sea Star	Arctic Surf-clam	Fat Gaper	Northern Horse-mussel	Softshell Clam	Washington Butterclam	Unknown Bivalve	Total
15-09	0	8	1	0	4	0	3	16
15-12	1	26	1	0	0	0	0	28
15-13	0	0	3	23	0	0	0	26
15-14	0	0	2	6	0	9	7	24
15-15	0	0	0	2	0	0	0	2
15-16	0	0	0	13	0	0	0	13
15-17	0	0	0	28	0	0	0	28
15-18	0	0	2	34	0	0	0	36
15-19	0	0	0	24	0	0	0	24
15-20	0	0	0	0	1	0	0	1
15-21	1	0	7	0	3	14	8	33
15-22	0	0	1	0	0	0	0	1
16-03	1	0	4	0	0	0	0	5
16-05	0	0	0	28	0	0	0	28
16-08	0	0	0	4	0	0	0	4
16-09	0	0	1	0	0	3	0	4
16-10	0	0	1	11	0	0	0	12
16-11	0	0	2	1	0	2	8	13
16-12	0	0	1	0	0	3	1	5
16-13	0	0	7	17	16	1	6	47
16-14	0	0	6	2	0	15	18	41
16-15	0	0	0	8	0	0	0	8
16-16	1	0	2	0	0	0	3	6
16-17	0	0	7	0	0	0	2	9
16-18	0	0	0	32	0	0	0	32
Totals	4	34	48	233	24	47	56	446

Notes:

[a] Sea otter numbers start with the last two digits of the year that sea otter was collected.

Chapter 2:

Metals in the stomach contents and brain, gonad, kidney, and liver tissues of subsistence-harvested northern sea otters (*Enhydra lutris kenyoni*) from Icy Strait, Alaska[1]

2.1. Abstract

The successful reintroduction and protection of sea otters (*Enhydra lutris*) to Southeast Alaska has led to a rapid rise in their population. As sea otters feed primarily on sessile prey, they are an excellent species for examining heavy metal contamination. The specific objectives of this study were to (1) determine concentrations of selected metals in different sea otter tissues; (2) determine whether selected metals biomagnify from stomach contents (i.e., the prey) to other sea otter tissues; (3) determine whether molar concentrations of selenium and mercury indicates an overall health benefit or risk to sea otters; and (4) determine if selected metals concentrations in sea otter tissues vary with sea otter size. Brain, gonad, kidney, and liver tissues, and whole stomachs were collected in April 2015 from fourteen freshly harvested sea otters in Icy Strait, Alaska. Samples were analyzed for arsenic, cadmium, copper, lead, total mercury, and selenium. Concentrations of metals varied significantly by tissue type, with livers and kidneys harboring the highest concentrations. Cadmium, copper, and selenium biomagnified in kidney and liver tissues. Total mercury biomagnified in all tissues analyzed. Lead and arsenic appeared to be readily excreted, with very low concentrations (most below 1.0 mg/kg wet weight). The results of this study represent baseline metals contamination data that can be used in monitoring the health of sea otter populations and the environments they inhabit.

[1] Brown, K.L., Atkinson, S., Furin, C.G., Mueter, F.J., Gerlach, R. (2020). Metals in the stomach contents and brain, gonad, kidney, and liver tissues of subsistence-harvested northern sea otters (*Enhydra lutris kenyoni*) from Icy Strait, Alaska. Manuscript in preparation for Marine Pollution Bulletin.

2.2. Introduction

Sea otters (*Enhydra lutris*) are widely known as a keystone species due to their significant influence in shaping the community structure of their ecosystem (Estes and Palmisano, 1974; Garshelis et al., 1986; Estes, 1990; Jessup et al., 2004). During the 18th century, Russian fur traders eradicated sea otters from their original range within Southeast Alaska (Kenyon, 1969; Jameson et al., 1982). In the absence of sea otters, lucrative dive fisheries for clam (*Bivalvia sp.*), sea urchin (*Echinoidea sp.*), sea cucumber (*Holothuroidea sp.*), and crab (*Brachyura sp.*) species were established in Southeast Alaska (USFWS, 1993). Successful reintroduction of sea otters carried out by the Alaska Department of Fish and Game from 1965 to 1972 (Jameson et al., 1982) has since resulted in a dramatic population increase to the Southeast Alaska region. Specifically, sea otters in Glacier Bay experienced a 44% annual rate of increase between 1999 and 2002 (Esslinger and Bodkin, 2009), slowing to a 20.6% annual increase between 2002 and 2012 (Tinker et al., 2019). The overall population of sea otters for all of Southeast Alaska is estimated at over 25,000 individuals, with over 8,000 of those residing in Glacier Bay (USFWS, 2014).

On average, sea otters consume nearly 25% of their overall body weight per day (Estes et al., 2003). Based on previous studies, sea otters in Alaska primarily feed on clams, mussels (*Mytilidae sp.*), crabs (Dungeness [*Cancer magister*] in particular), sea urchins, and sea cucumbers, with clams comprising over 70% of the diet in soft-bottom habitat (Garshelis et al., 1986; Kvitek et al., 1993; Wolt et al., 2012; Larson et al., 2013), and bivalves making up more than 90% of the diet in Icy Strait, Southeast Alaska (Brown et al., 2019). Compared to other marine mammals which migrate large distances, most sea otters live within reasonably small home ranges, and along with their primary prey, are relatively sedentary; therefore, their contaminant loads likely reflect contamination of the local environment (Bacon et al., 1999; Comerci et al., 2001; Jessup et al., 2004; Kannan et al., 2008; Brancato et al., 2009). Sea otters target sessile prey which filter feed on sediments and detritus (or kelp [Order Laminariales] in the case of sea urchins), and can ingest and concentrate a variety of environmental contaminants (Bacon et al., 1999; Jessup et al., 2004; Carswell et al., 2015). The majority of prey species eaten by sea otters are

also consumed by humans and are considered important species to commercial, recreational, and subsistence fisheries (Estes, 1990; USFWS, 1993; Jessup et al., 2004).

Sea otters are protected under the Marine Mammal Protection Act of 1972 (16 U.S.C. § 1361 *et seq.* MMC, 2007), and under the Endangered Species Act for northern sea otters (*E. l. kenyoni*) in Southwest Alaska (70 F.R. 46366-46386, 2005) and for southern sea otters (*E. l. nereis*) in California (42 F.R. 2965-2968, 1977). Northern sea otters, the primary focus of the present study, occupy the middle-range of the North Pacific rim from northern California to the Aleutian Islands in Alaska and in the Commander Islands at the westernmost end of the Aleutian Archipelago. This population of sea otters in U.S. waters is managed by the United States Fish and Wildlife Service (USFWS). Only Alaska Natives are allowed to hunt sea otters as part of their tradition and for subsistence purposes (50 C.F.R. § 18.23, 2000), with the exception of strictly prohibited hunting within Glacier Bay National Park and Preserve (USWFS, 1993; Esslinger and Bodkin, 2009).

Given the strong conservation measures in place for the species, research on sea otter tissues is logistically difficult and many contaminant studies on sea otters must utilize stranded sea otter carcasses (Morejohn et al., 1975; Kubota et al., 2001; Kannan et al., 2006). While this is an adequate and advantageous way to opportunistically collect scientific data, it is challenging to analyze such samples for contaminant concentrations due to unknown prior conditions of the animal. The cause of death may well be attributed to abnormally high contaminant concentrations for the animal, or illness that suppresses or eliminates appetite. Contaminant concentrations measured in diseased, starving, or just generally unhealthy sea otters are unlikely to accurately represent the true body burden of healthy animals from a robust population. Evaluating how contaminants bioaccumulate and are distributed in tissues of top trophic level predators provides an indication for potential exposure to humans and demonstrates how these keystone species act as sentinels or indicators of local coastal ecosystem health (Jessup et al., 2004; Moynahan and Johnson, 2008). As humans consume seafood items which are also heavily consumed by sea otters, contaminant levels in sea otter tissues offers a glimpse at what humans may be exposed to, albeit on a smaller scale considering that humans are eating a small fraction of the same foods that sea

otters consume daily. While limited, there are reports of Alaska Natives consuming sea otter meat (M. Gho, personal communication, 2018), and any contaminants within that meat would presumably bioaccumulate into the consumer's body.

For the present study, 'heavy metals' refers to arsenic, cadmium, copper, lead, total mercury, and selenium. Heavy metal exposure and toxicity from the marine environment are bioaccumulative, meaning they get absorbed from the diet and surrounding waters faster than the organism can eliminate or excrete them (Debruyn and Gobas, 2006; Daley et al., 2014). They also tend to have a slow rate of degradation. Some metals have the potential to biomagnify up the food chain, meaning that the top-level consumer ends up with a higher contaminant load than that of the prey they ingested (Cardoso et al., 2014; Daley et al., 2014). While all of these metals are naturally occurring, they have also all been found associated with mining, and Southeast Alaska has a fairly significant mining history (MacKevett et al., 1971). Several mineral deposits sampled in the Glacier Bay Monument in 1966 were found to have relatively high concentrations of arsenic, cadmium, copper and lead metals (MacKevett et al., 1971), all of which were also examined in the present study. Though selenium was not analyzed in the Glacier Bay study, selenium has been shown to be associated with glacial silt deposits in other areas of the United States (Searight and Moxon, 1945). With the receding of the glaciers in Glacier Bay, these mineral deposits may have been released into the water which drains directly into Icy Strait. It is important to evaluate the levels of naturally occurring metals in these areas considered by many to be pristine (Bacon et al., 1999; Carswell et al., 2015).

Heavy metals have been widely studied in sea otters from Russia, California, Washington, and Southcentral and Southwest Alaska (Comerci et al., 2001; Kubota et al., 2001; Kannan et al., 2006, 2008). However, relatively few studies have examined contaminants in the Southeast Alaska sea otter population (Comerci et al., 2001). Previously conducted studies appear to only evaluate kidneys and livers, and in one study, whole blood. Additionally, a majority of sea otters used in such studies were beach-cast carcasses, and therefore not representative of metals concentrations in the healthy population. Heavy metals including arsenic, cadmium, lead, copper, selenium, and mercury are ranked as a priority in public

health significance due to their high degree of toxicity, classification as carcinogens, and potential for inducing multiple organ damage even at low levels of exposure (USEPA, 2000). Determining where contaminants get distributed in the body is vital to interpreting their impacts on the physiology of an organism. Yet there have been apparently no studies conducted on metals concentrations in the brain and gonad tissues of sea otters in any region, or on the distribution of contaminants to various sea otter tissues, particularly for the Southeast Alaska population of sea otters.

The overall goal of the present study was to examine metals contamination in apparently healthy sea otters, in order to obtain baseline data for the Glacier Bay/Icy Strait coastal marine ecosystem of Southeast Alaska. The specific objectives were to (1) determine concentrations of selected metals in different sea otter tissues (brain, gonad, kidney, and liver); (2) determine whether selected metals biomagnify from stomach contents (i.e., the prey) to other sea otter tissues; (3) determine whether molar concentrations of selenium and mercury indicates an overall health benefit or risk to sea otters; and (4) determine if selected metals concentrations in sea otter tissues vary with sea otter size. Because Glacier Bay waters tend to be considered "pristine" (Bacon et al., 1999; Carswell et al., 2015), it was expected that the metals concentrations for this study would all be low, especially when compared to studies conducted in other regions or for populations of sea otters that are in decline.

2.3. Materials and Methods

2.3.1. Sample Collection

The samples for this study were opportunistically collected from recently killed, but otherwise seemingly healthy sea otters that were part of Alaska Native subsistence hunts. Fourteen sea otters (4 females and 10 males) were shot and collected by Alaska Native subsistence hunters in the waters of Icy Strait, just outside of Glacier Bay, Alaska in April 2015 (Figure 2.1). Samples for this research were collected all in one day (Table 2.1). In the field, standard length and axillary girth of each sea otter were measured to the nearest inch using a flexible vinyl measuring tape. Standard length was measured from the tip of the nose to the tip of the tail along a flat surface with the sea otter on its back. Axillary girth was measured around the body of the sea otter at the axilla. Weights were measured to the nearest pound using

a hanging scale. Brain, gonad, kidney, and liver tissues, and whole stomachs were collected from each of the freshly dead sea otters. Brain samples from 2 of the 14 sea otters were not collected. Sea otter hunters aim for the head when hunting and two of the sea otters did not have enough brain tissue to collect post-mortem. One of the samples was lead-contaminated (most likely due to a bullet fragment in the sample) and therefore the lead value in the brain of sea otter #3 was considered an outlier and removed from analyses. The rest of the samples collected from sea otter #3 were assumed to be unaffected. A premolar or incisor tooth was removed from the skull in the field for age analysis. All the samples were collected in the field, stored chilled with ice packs, and brought back to the laboratory at the University of Alaska Fairbanks (UAF) Juneau Center of the College of Fisheries and Ocean Sciences (JC-CFOS) for further processing. Samples were kept chilled after field collection and frozen before being shipped to the State of Alaska Environmental Health Laboratory (EHL) in Anchorage for contaminants analysis. A subsample (approximately 20 grams) of the contents from 13 of the 14 stomachs (one stomach was empty) was chosen for contaminant analysis as a representative snapshot of contaminant levels in sea otter prey. Stomach fluid was separated from the stomach contents prior to submission to the EHL for analysis. The rest of the stomach contents and whole stomachs were retained at the UAF JC-CFOS for use in a companion study (Brown et al., 2019).

2.3.2. Metals Analyses

The EHL analyzed all samples for arsenic, cadmium, copper, lead, and selenium using EPA Method 6020A and for total mercury using EPA Method 7473 (following Farrugia et al., 2015). For stomachs, fluids were separated from solids and only the solids were processed for analysis. Total mercury was determined using a DMA-80 (Milestone Inc., Shelton, CT, USA) direct mercury analyzer. This method only measured total mercury and did not differentiate between different forms of mercury. Method Detection Limits (MDLs) and Reporting Limits (RLs) are lab-specific and were provided by the EHL. For mercury, the MDL was 0.0048 mg/kg and the RL was 0.01 mg/kg. The laboratory control sample recovery criteria were 71-124% for mercury; all percent recoveries were within the respective method limits. Arsenic, cadmium, copper, lead, and selenium were measured using an Elan DRC II

(PerkinElmer Inc., Waltham, MA, USA) inductively coupled plasma mass spectrometer (ICP-MS). This method was used to measure the total amount of each element and did not differentiate between different forms of the elements. The MDLs were 0.0077 mg/kg for arsenic, 0.0014 mg/kg for cadmium, 0.05 mg/kg for copper, 0.01 mg/kg for lead, and 0.01 mg/kg for selenium. The RLs were 0.05 mg/kg for arsenic, 0.05 mg/kg for cadmium, 0.05 mg/kg for copper, 0.05 mg/kg for lead, and 0.25 mg/kg for selenium. The laboratory control sample recovery criteria were 80-130% for arsenic, 75-125% for cadmium, 75-125% for copper, 75-125% for lead, and 90-140% for selenium; all percent recoveries were within the respective method limits. Standard EHL Quality Assurance/Quality Control protocols were followed for all samples. All results were expressed on a wet weight basis.

Concentrations of metals that resulted as non-detect (ND) were evaluated at half their MDL (Helsel, 2012). Only lead and cadmium returned non-detect results (~27% and 3%, respectively). All other metals yielded detectable concentrations (Table 2.2). Liver results returned zero NDs. Brain had ~8% NDs for cadmium and ~42% NDs for lead. Kidney had ~21% NDs for lead. Gonads had ~64% NDs for lead. Stomach contents had ~8% NDs for both lead and cadmium. Concentrations of metals that resulted in values between the RL and the MDL for that metal (considered estimates, n=10) were treated as measured values (Table 2.2; Helsel, 2012).

2.3.3. Moisture Content Analysis

Leftover homogenized sea otter tissue was shipped back to UAF JC-CFOS for moisture content analysis. In brief, the analysis protocol was as follows. Pans were dried in a VWR International E1310 model oven prior to obtaining pan weight. Weighed subsamples of approximately 10 g of tissue was added to the pan. Pans with tissue were dried between 100-110 degrees Celsius overnight (at least 24 hours). Pans were then weighed, placed back in the oven to dry again, and re-weighed after an hour. These weight measurements agreed within 4%. Percent moisture was then calculated using the following formulas:

$$\%\, Solids = \frac{(X_1 - X_2)}{X_3} \times 100,$$

where X_1 is the weight of the dry tissue plus the weight of the pan, X_2 is the weight of the pan (without any tissue), and X_3 is the wet weight of the tissue, and

$$\% \, Moisture = 100 - \% \, Solids.$$

Duplicates of each sea otter tissue were also dried and analyzed for moisture content. Duplicate relative percent difference (RPD) was calculated as:

$$RPD = \left(\frac{|X_1 - X_2|}{\frac{(X_1 + X_2)}{2}} \right) \times 100,$$

where X_1 is the percent solids of the sample (i.e., the pan weight plus first measured dry weight), and X_2 is the percent solids of the duplicate sample (i.e., pan weight plus final measured dry weight). All measurements were weighed to the nearest 0.01 g using an OHAUS PA1502 scale. Wet weights were converted to dry weights using the formula:

$$[DW]_i = [WW]_i \times \left(\frac{100}{100 - \% \, Moisture} \right)$$

where $[DW]_i$ is the mean concentration of metal i in dry weight (mg/kg) as converted from the wet weight (mg/kg) mean concentration of that same metal i ($[WW]_i$) (AOAC, 2002; Lusk et al., 2005).

There was only enough wet liver tissue from one sea otter and wet kidney tissue from two sea otters to analyze for moisture content (Table 2.3). The percent moisture content result for the one liver was then used to calculate dry weights for all liver metal concentrations. The percent moisture content results for the two kidneys was averaged and that average was then used to calculate dry weights for all kidney metal concentrations. The average of the three tissues analyzed for moisture content (one liver and two kidneys) was 74.67%±4.15% (Table 2.3). While no values were found in the literature for percent moisture of gonads and stomach contents, one study noted that concentrations of mercury in brain tissue per unit wet weight could be converted to concentrations per unit dry weight by multiplying the wet weight values by a factor of 4 (Scheuhammer et al., 2015); or in other words, brain tissue had 75% moisture content. With a paucity of information regarding moisture content in all the tissues used for the

present study, a moisture content of 75% was chosen and applied to the brains, gonads, and stomach contents so that concentrations based on dry weights could be calculated for all metals in those tissues.

2.3.4. Age Analysis

Teeth were analyzed at Matson's Tooth Aging Laboratory (Manhattan, Montana) as was also done in Hutchinson et al. (2015) for the same population of sea otters. Growth layers in the cementum of the extracted premolar (standard tooth) or incisor (non-standard tooth) were used to obtain individual sea otter ages, based on one growth layer per year. Two of the fourteen teeth analyzed for age were noted as having been broken with missing cementum; however, Matson's Laboratory was still able to accurately determine age for those individuals. One of the fourteen teeth from the sea otters collected was noted by Matson's Laboratory as being a non-standard tooth; however, again Matson's Laboratory determined that age analysis was not affected. Six total teeth were given an age range in addition to the age result provided. These six teeth were assigned a lower reliability index, indicating that there is histological evidence to support the age result provided and the correct age is expected to fall within the age range given.

2.3.5. Data Analyses

Statistical analyses were conducted using R version 3.4.4 (R Core Team, 2018). To differentiate from areas of Southeast Alaska outside this project's study area, sea otters from the present study will be referred to as Icy Strait sea otters. Means and standard deviations were calculated for each metal by tissue type. Random effects models were used to describe metals concentrations in sea otter tissues. Sea otter sex was not significant to any of the models tested for each metal ($p>0.05$) and low sample sizes would not allow for estimating separate effects; therefore, males and females were combined for analyses. Preliminary analyses suggested a curvilinear relationship between length and mercury concentrations, therefore a quadratic term for length was included in the full model. A log-transformation of each of the metals concentrations was required to attain approximate normality and equal variances. In order to account for animal specific differences, a random effects model was fit to the data for comparing log-

transformed metals concentrations (mg/kg wet weight) across sea otter tissue types while accounting for differences in metals concentrations with length (centimeters). The model is denoted as follows:

$$y_{ik} = \alpha + a_k + \mu_i + \beta_1 L + \beta_2 L^2 + \varepsilon_{ik}, \quad \text{(Model 1)}$$

where y_{ik} is the (log-transformed) metal concentration for animal k in tissue type i, α is the intercept, a_k is the random effect associated with animal k, μ_i is the effect of tissue type i on metal concentration (i.e., the difference in concentration between tissue type i and the intercept), β_1 and β_2 are coefficients that describe the estimated changes in metal concentrations with sea otter length L, and ε_{ik} is the residual for animal k and tissue type i, which is assumed to be normally distributed with mean zero and variance σ_ε^2. The above model was compared to a model with length as a linear term:

$$y_{ik} = \alpha + a_k + \mu_i + \delta L + \varepsilon_{ik}, \quad \text{(Model 2)}$$

a model that did not include any length term:

$$y_{ik} = \alpha + a_k + \mu_i + \varepsilon_{ik}, \quad \text{(Model 3)}$$

and to a null model that did not include the effect of tissue type while still accounting for possible differences in average metals concentrations among animals:

$$y_{ik} = \alpha + a_k + \varepsilon_{ik}. \quad \text{(Model 4)}$$

Sea otter age in place of length was also explored for each of these models. Sea otter age was positively correlated with sea otter length. Although age demonstrated a nearly identical relationship to each of the metals as length did, length was ultimately chosen for final analyses as it was deemed to be a more accurate measurement than age. Furthermore, models involving interactions between linear length and tissue type were considered, but the interaction term was generally not significant to the model ($p>0.05$) and there were no *a priori* reasons to believe that there should be interactions, so that model was dropped from the list of models presented herein. The best model for each metal was selected based on the small-sample Akaike Information Criterion (AICc) and model weights (following Zaleski et al., 2014; Table 2.4; main model effects, Table 2.5). After adjusting for multiple comparisons using Tukey's

contrasts, pairwise differences were evaluated for metals concentrations in each pair of tissues (Figure 2.2).

Biomagnification factors (BMFs) were calculated as:

$$BMF = \frac{Metal_T}{Metal_{SC}},$$

where $Metal_T$ is the mean concentration of the selected metal in mg/kg wet weight of the brain, gonads, kidney, or liver tissues, and $Metal_{SC}$ is the mean concentration of the selected metal in mg/kg wet weight of the stomach contents (adapted from Cardoso et al., 2014; originally from Hoekstra et al., 2003). Biomagnification factors greater than 1 indicated accumulation from the stomach contents (i.e., the prey) to higher level tissues. Values less than 1 suggest element elimination or trophic transfer interruption (the inefficiency of a metal to transfer to the next higher trophic level; Hoekstra et al., 2003).

The Health Benefit Value of Selenium (HBV_{Se}) was adapted to the present study and calculated as a measure of mercury toxicity mitigation by selenium, based on the methods of Ralston et al. (2015). After converting concentrations (mg/kg wet weight) of selenium (Se) and total mercury (THg) to molar concentrations, the HBV_{Se} for each tissue was calculated as:

$$HBV_{Se} = ([Se - THg]/Se) \times (Se + THg).$$

The mean HBV_{Se} value (± standard deviation) was then calculated for each tissue type. A negative HBV_{Se} value indicates a surplus of mercury and an overall health risk, whereas a positive value indicates an overall health benefit (Ralston et al., 2015), with the magnitude being proportional to the benefit or risk.

2.4. Results and Discussion

2.4.1. Variability in Metals by Tissue Type and Length

Concentrations of arsenic, cadmium, total mercury, and lead differed significantly among tissues and were significantly related to sea otter length (Figure 2.3). Variability in total mercury was best described by the full model (Model 1), which included a quadratic term for length, while arsenic, cadmium, and lead metals increased linearly with length (Model 2; Table 2.4). Concentrations of copper and selenium were not significantly related to length but differed by tissue type (Model 3; Table 2.4). It is

important to note that for arsenic, Models 1, 2, and 3 are barely distinguishable from each other (ΔAICc<1), indicating that arsenic has a very weak relationship with sea otter length. Pairwise comparisons suggested that metal concentrations differed significantly between tissue types (Tukey's contrasts: $p<0.05$; Figure 2.2), with the exception of lead in brain-kidney and kidney-liver, and selenium in gonads-liver. In general, arsenic and lead had the highest concentrations in stomach contents, cadmium and selenium were highest in the kidneys, and copper and total mercury were highest in livers. Mean concentrations of metals occurred in the following orders: 1) arsenic, stomach contents > kidney > liver > gonads > brain; 2) cadmium, kidney > liver > stomach contents > gonads > brain; 3) copper, liver > kidney > stomach contents > brain > gonads; 4) lead, stomach contents > liver > kidney > brain > gonads; 5) mercury, liver > kidney > brain > gonads > stomach contents; and 6) selenium, kidney > liver > stomach contents > gonads > brain. While brains and gonads had the lowest metals concentrations of any tissue, the metal within the brain that was highest was copper, and within the gonads was selenium.

2.4.2. Biomagnification Factors

Biomagnification factors for arsenic and lead were all below 1, indicating no biomagnification of these metals was taking place in sea otters. Cadmium, copper, and selenium biomagnified in kidney and liver tissues, but not to the brain or gonad tissues. Only total mercury demonstrated biomagnification from the stomach contents to all higher-level tissues (Table 2.6). Biomagnification of mercury, particularly methylmercury, has been demonstrated for several species, including fish and shellfish, mammals such as mule deer (*Odocoileus hemionus*), moose (*Alces alces*), caribou (*Rangifer tarandus*), marten (*Martes martes*), polecat (*Mustela putoris*), red fox (*Vulpes vulpes*) and various species of rabbits (*Oryctolagus cuniculus*), as well as marine mammals like ringed seals (*Pusa hispida*) (Eisler, 1987). The results of the present study provide further evidence that mercury biomagnifies in sea otter food webs. Since methylmercury could not be differentiated from total mercury, the extent that methylmercury alone (the most toxic form) was biomagnified cannot be fully assessed, but would be worthy of additional research.

2.4.3. Literature Comparisons for Metals Results

In order to facilitate literature comparison (Table 2.7), further discussion will refer to this study's calculated dry weight values for metals concentrations (except where otherwise noted). Since metals concentrations in the brains, gonads, and stomach contents are not represented for sea otters in the published literature, and since brain tissue and gonads had considerably lower metals concentrations compared to other tissues in the present study, discussion will primarily focus on livers and kidneys. Metals concentrations in the stomach contents were presumed to reflect contaminant loads of the ingested prey rather than that of the sea otter stomach, as the stomach contents, which were primarily bivalve species (Brown et al., 2019), were the tissues that were analyzed. Data on threshold values for deleterious effects of metals concentrations on sea otters are not documented, and therefore threshold values will be derived from other species (as closely related to sea otters as possible) for comparison.

2.4.3.1. Arsenic

Concentrations of arsenic ranged from 0.05 to 3.01 mg/kg wet weight and were highest in the stomach contents (57.1% proportionally), followed by the kidneys (22.6%), with considerably lower concentrations in the liver (9.5%) and gonads (7.4%), which in turn were considerably higher than concentrations in the brain (3.4%) (Figure 2.2; Tables 2.7 and 2.8). Mean arsenic concentrations of both the kidneys and the livers were very similar across sea otters from Icy Strait, Alaska (present study) and those studied in Southeast, Southcentral, and Southwest, Alaska (Figure 2.4; Comerci et al., 2001). Sea otters from Washington had livers with nearly identical wet weight concentrations of arsenic as sea otters from Icy Strait (Brancato et al., 2009), while Baja California sea otters had almost three times as much arsenic in their livers than those of the present study (Kubota et al., 2001). Arsenic concentrations in the study were related to sea otter body length, in that the shortest and longest sea otters had the lowest amount of arsenic levels, which was particularly evident in the stomach contents and kidneys (Figure 2.3).

As confirmed by this study, arsenic does not biomagnify up the food chain (Table 2.6; Eisler, 1988a; Kubota et al., 2001). In fact, mean arsenic concentrations in the stomach contents were significantly higher than in other tissues in the present study (Tukey's contrasts: $p<0.05$), indicating the

efficacy with which sea otters process and excrete arsenic from their diet (Eisler, 1988a). While arsenic can cross into the placenta and lead to fetal death in many species of mammals, a lack of arsenic can cause impaired reproduction, insufficient growth, and decreased survival. Long-term arsenic exposure has been associated with hearing loss, brain wave irregularities, and damage to the liver, kidneys, and heart. Concentrations of arsenic generally tend to be low (<1.0 mg/kg wet weight), but are higher in the marine environment where arsenic is primarily in its organic form and does not pose much, if any, risk to the organism (Eisler, 1988a; Taylor et al., 2017). Hence, most arsenic in shellfish, which is the primary prey source of sea otters from the area of the present study (Brown et al., 2019), is in the organic form, is nontoxic, and is unlikely to be causing any deleterious effects to the health of the sea otter population from the present study.

2.4.3.2. Cadmium

Concentrations of cadmium ranged from non-detect to 81.10 mg/kg wet weight, and were highest in the kidney (68.6% proportionally), followed by the liver (27.4%), which were both higher than the stomach contents (2.5%), gonads (1.2%), and brain (0.3%) (Figure 2.2; Tables 2.7 and 2.8). Other research conducted throughout Alaska also found greater concentrations of cadmium in sea otter kidneys as compared to livers (Figure 2.4; Comerci et al., 2001). This pattern coincides with findings in other marine mammals, including polar bears (*Ursus maritimus*) and ringed seals (AMAP, 1998). However, mean concentrations of cadmium in the kidneys of Icy Strait sea otters were 7 to 9 times greater than that of sea otters from Southeast, Southcentral and Southwest Alaska, and from Russia (Comerci et al., 2001). Deleterious effects of cadmium toxicity include delayed growth, physiological malformations, gene mutation, respiratory disruption, impaired reproduction, cancer development, and renal dysfunction (Eisler, 1985a; Law, 1996). Yet, the concentrations found in the present study are still below even the low-end range of effects thresholds for cadmium in undefined marine mammal species' kidneys (50-400 mg/kg wet weight) and livers (20-200 mg/kg wet weight) (Law, 1996). Therefore, it is unlikely that the sea otters in the present study are experiencing any negative effects due to cadmium.

In the present study, cadmium concentrations increased with sea otter body length, particularly in the kidneys and livers (Figure 2.3). Length increases to 155 cm by age 5 and then levels off (as also demonstrated by Hutchinson et al., 2015), and therefore cadmium concentrations were lowest in the youngest sea otters and highest in sea otters aged 5 and older. This is not entirely surprising as cadmium is well-known to increase with age in marine mammals (Eisler, 1985a; Law, 1996; AMAP, 1998), which has been demonstrated for harbor seals (*Phoca vitulina*; Miles et al., 1992), northern fur seals (*Callorhinus ursinus*; Goldblatt and Anthony, 1983), and Pacific walruses (*Odobenus rosmarus divergens*; Warburton and Seagars, 1993).

2.4.3.3. Copper

Concentrations of copper ranged from 0.83 to 38.30 mg/kg wet weight and were highest in the liver (57% proportionally), followed by the kidneys (22.9%), stomach contents (10.4%), brain (6.9%), and gonads (2.8%) (Figure 2.2; Tables 2.7 and 2.8). Copper concentrations did not demonstrate a relationship with sea otter body length (Table 2.4). In kidney tissue, mean copper concentrations were twice as high or greater in Icy Strait sea otters as compared to the published studies (Comerci et al., 2001). In liver tissue, the highest copper concentrations were measured in Prince William Sound sea otters (Figure 2.4; Kannan et al., 2008), followed by sea otters captured in California, which had roughly half the concentrations of Prince William Sound sea otters (Kannan et al., 2008). Mean copper concentrations in Icy Strait sea otters were less than half of the highest reported copper concentrations for sea otter livers (Prince William Sound) and were very closely aligned with results found in Southcentral Alaska and Washington sea otters (Comerci et al., 2001; Kannan et al., 2008; Brancato et al., 2009). The lowest concentration of copper in sea otter livers was found in Southeast Alaska, Adak Island, Alaska, and Kamchatka, Russia (Comerci et al., 2001; Kannan et al., 2008).

Copper is considered one of the most toxic heavy metals in both freshwater and the marine environment. Deleterious effects of copper toxicity include delayed or reduced growth, inhibited reproduction, disrupted kidney and liver function, decreased survival, and death (Eisler, 1998). Copper is an essential micronutrient. A deficiency in copper is harmful and can cause many of the same effects as

having an excess of copper, as well as induce blood disorders such as anemia and lesions in the cardiovascular, skeletal, and central nervous systems (Eisler, 1998). Copper concentrations in marine mammal tissues typically do not exceed 44 mg/kg dry weight except in the liver (Eisler, 1998). In general, concentrations of copper in marine mammal livers do not exceed 116 mg/kg dry weight; however, liver concentrations of copper have been shown to be as high as 146 mg/kg dry weight in polar bears, and 1,200 mg/kg dry weight in manatees (*Trichechus sp.*; Eisler, 1998). To compare, copper concentrations in sea otter livers from the present study ranged from 33.96 mg/kg to 133.67 mg/kg dry weight, with an average of 80.28 mg/kg (Table 2.7). Risk levels have not been established for copper in sea otters, but according to the general information available, it would appear sea otters in the present study fall within the range of copper concentrations found in other marine mammals. Therefore, it is unlikely copper is causing any harmful effects to sea otters in Icy Strait.

2.4.3.4. Lead

Concentrations of lead ranged from 0.01 to 6.47 mg/kg wet weight and were highest in the stomach contents (68.1% proportionally), with lower concentrations in the liver (14.6%), kidneys (9.1%), brain (6.4%), and gonads (1.8%) (Figure 2.2; Tables 2.7 and 2.8). Ranges of lead levels in the present study were close to those found in other studies for sea otter kidneys and livers (Figure 2.4; Comerci et al., 2001; Kannan et al., 2006, 2008). Lead concentrations increased with increasing sea otter length for all tissues, with the most pronounced increase in the liver (Figure 2.3). Lead concentrations have been shown to increase with age in marine mammals, except in northern fur seals (Goldblatt and Anthony, 1983; Law, 1996). As sea otter age was positively correlated with sea otter length in the present study, it makes sense that a positive relationship between sea otter length and lead concentrations was also found (Figure 2.3).

Lead is considered a non-essential element, with most of its chemical forms being toxic (Goldblatt and Anthony, 1983; Eisler, 1988b). The deleterious effects of lead toxicity include reduced growth, developmental disruption, anemia, blindness, neurological disorders, renal dysfunction, impaired reproduction, decreased appetite, and weight loss (Ma, 1996). Lead concentrations in the present study

were typically low in all tissues (below 1.0 mg/kg wet weight; except in the stomach contents of sea otter ID #12, which was 6.47 mg/kg wet weight; Table 2.2), and were below effects threshold levels established for mammals for both livers (30 mg/kg dry weight) and kidneys (90 mg/kg dry weight) (Ma, 1996). Based on these results, lead is unlikely to be causing any harmful effects to Icy Strait sea otters.

2.4.3.5. Mercury

Concentrations of mercury in Icy Strait sea otter tissues ranged from 0.01 to 2.26 mg/kg wet weight and were highest in the liver (59.8% proportionally), followed by the kidneys (30.6%), with considerably lower concentrations in the brain (4.8%), gonads (3.4%), and stomach contents (1.4%) (Figure 2.2; Tables 2.7 and 2.8). Mercury concentrations were generally low in all sea otter tissues (all but four results were <1.0 mg/kg wet weight; Table 2.2). While mean mercury concentration of the kidneys in Icy Strait sea otters was approximately double the amount found in other Southeast and Southcentral Alaskan sea otter kidneys, and were 30 times greater than mean mercury concentrations in sea otter kidneys from Southwest Alaska, Icy Strait sea otter kidney concentrations still fell within the ranges reported for Southeast, Southcentral, and Southwest Alaska sea otters (Comerci et al., 2001). For liver tissues, concentrations of mercury in Icy Strait sea otters closely matched those of sea otters in Southcentral Alaska (Comerci et al., 2001), and were about three to four times higher than concentrations in sea otters from Southeast Alaska, Southwest Alaska (including Adak Island), and Kamchatka, Russia (Figure 2.4; Comerci et al., 2001; Kannan et al., 2008), but are considerably lower than concentrations in sea otters from Prince William Sound, Alaska, Washington, and California (Kannan et al., 2006, 2008; Brancato et al., 2009).

Mercury concentrations had a bimodal distribution with sea otter length, indicating that total mercury levels were higher in the shortest and longest sea otters (Figure 2.3; Table 2.4). Sea otter length demonstrated a curvilinear relationship with age. In general, the shortest sea otters were also the youngest, with length leveling off around 155 cm at around 5 years of age (as also demonstrated by Hutchinson et al., 2015). It was originally expected that mercury concentrations would be highest in the longest animals, assuming that the longest animals would also be some of the oldest and would consume much greater

amounts of mercury-contaminated prey species (thereby accumulating more mercury over the years). However, sea otter age and the number of prey species inside the stomachs of sea otters (used as a general index of the amount of prey consumed), were found to be unrelated (Brown et al., 2019), indicating that sea otters of all ages (and therefore all lengths) are consuming the same number of prey items at any one time. It is possible that like other marine mammals, such as Pacific walruses (Warburton and Seagars, 1993), grey seals (*Halichoerus grypus*; Habran et al., 2013), Steller sea lions (*Eumetopias jubatus*; Rea et al., 2013), and harbor seals (Noël et al., 2016), sea otter mothers may offload some of their mercury contamination to pups either during gestation when mercury can pass through the placenta, or in nursing (Debruyn and Gobas, 2006), thereby possibly accounting for the higher mercury levels seen in the shortest animals (which were also some of the youngest). However, it is important to note that sea otter pups/juveniles are typically considered to be roughly 0 to 2 years of age (Schneider, 1973; Garshelis et al., 1986; Brown et al., 2019), and the present study had only two sea otters estimated to be 1 year old (sea otter IDs #2 and #8), and four sea otters estimated to be 2 years old (sea otter IDs #4, #7, #9, and #14) (Table 1).

Deleterious effects of mercury toxicity include birth defects, delayed or impaired development, reduced growth, impaired reproduction, brain and liver damage, deterioration of vision and hearing, adverse changes in blood chemistry and metabolism, and death (Eisler, 1987; AMAP, 1998). Smaller animals are noted to be more susceptible to mercury poisoning than larger animals (Eisler, 1987), although it is unclear as to why. In marine mammals, threshold effects of mercury have been established at 60 mg/kg wet weight for livers (Law, 1996) and 1.1 mg/kg wet weight for kidneys (Eisler, 1987). Mean mercury concentrations in the kidneys and livers of Icy Strait sea otters were considerably below these established threshold levels. Only one sea otter from the present study had a mercury level in the kidney that was greater than the documented threshold (1.43 mg/kg wet weight in sea otter ID #11; Table 2.2). This same sea otter (sea otter ID #11) had the highest mercury concentration in its liver (at 2.26 mg/kg wet weight) compared to the mercury levels of all other sea otter livers in the present study (Table 2.2). Despite sea otter #11 having a higher mercury level in both its kidney and liver, the rest of the sea otters

in the present study had relatively low mercury concentrations and it is unlikely that mercury is causing any harmful effects to these sea otters.

2.4.3.6. Selenium

Concentrations of selenium ranged from 0.70 to 7.83 mg/kg wet weight and were highest in kidneys (44.7%), with lower concentrations in the liver (16.1%), stomach contents (15%), gonads (14.7%), and brain (9.5%) (Figure 2.2; Tables 2.7 and 2.8). Selenium concentrations did not demonstrate a relationship with sea otter body length (Table 2.4). In livers, the highest concentrations of selenium were found in sea otters from Washington (Brancato et al., 2009), followed very closely by Icy Strait sea otters and then by sea otters in Southcentral Alaska, which in turn was approximately two to three times the concentration of selenium found in sea otters from Southwest and Southeast Alaska, respectively (Figure 2.4; Comerci et al., 2001). It is challenging to evaluate the significance of selenium values to the health of an animal, as tolerance to selenium can vary greatly by species (Eisler, 1985b), and it is difficult to compare marine and terrestrial environments, particularly for selenium; however, threshold effects of selenium concentrations have not been established for sea otters. Based on the effects threshold level established for livers in terrestrial mammals (7 mg/kg dry weight), sea otters in the present study would be at risk or may have apparent hepatic lesions (AMAP, 1998). Although no lesions were noted for the livers collected in the present study, this was not a characteristic specifically screened for in the field or at the laboratory. However, if hepatic lesions had been readily apparent, they most likely would have been noted since field notes did indicate irregularities observed in other tissues (such as possible cysts seen on one kidney). In kidneys, mean selenium concentrations in Icy Strait sea otters were consistently three to four times higher when compared to sea otters all throughout Alaska and in Russia (Comerci et al., 2001).

Perhaps the high levels of selenium found in the present study can be at least partially attributed to the geographical location where samples were collected. Icy Strait waters are directly fed by the tidewater glaciers of Glacier Bay, which historically covered all of Icy Strait. Elevated selenium concentrations have been discovered in all glacial and associated deposits in areas of South Dakota and Minnesota, up to 5.38 parts per million (ppm) selenium (Searight and Moxon, 1945). Selenium poisoning

has been observed in horses (*Equus caballus*), cattle (*Bos taurus*), and sheep (*Ovis aries*) of South Dakota demonstrated by the animals via erratic behaviors, abnormal hoof development, vision impairment, paralysis, and post-mortem liver lesions (Durrell and Cross, 1944). Selenium concentrations in moose found dead in Minnesota were shown to be at an "adequate to high" level (0.86–4.28 mg/kg dry weight) in 65.5% of moose livers and to be at a chronic toxicity level (>4.29 mg/kg dry weight) in 16% of moose livers (n=81) (Custer et al., 2004). It is possible that Icy Strait has elevated concentrations of selenium due to its high tidewater glaciation, where multiple glaciers' termini affront marine waters and are most likely depositing selenium directly into those waters; as compared to selenium concentrations in sea otters from Russia, California, Washington, and Southcentral and Southwest Alaska areas, which are relatively non-glaciated in comparison. Higher concentrations of selenium are likely either ingested directly through the marine environment or via sea otters' molluscan prey items, which bioaccumulate selenium through their feeding mode. Considering the interactions that selenium has with other elements in the body, it is difficult to understand how selenium levels found in sea otters from the present study are affecting sea otter health in the Icy Strait area. It is likely though, that because the sea otter population in this area is continually rising, these sea otters are not experiencing any deleterious effects due to selenium.

2.4.4. Selenium Health Benefit Values

Selenium's ability to mitigate the negative effects of mercury has been well studied (Ralston et al., 2015). Selenium health benefit values were positive in all sea otter tissue types analyzed in the present study, with the highest HBV_{Se} in kidneys, followed with almost equal values by liver, stomach contents, and gonads, and lastly by brain (Table 2.6). Positive values indicate concentrations of selenium are greater than mercury concentrations, and that selenium levels are protecting those tissues against mercury toxicity (Ralston et al., 2015), with the kidneys receiving maximum benefits. However, when examining selenium levels alone, sea otters in the present study had concentrations greater than the effects threshold level established for livers in terrestrial mammals (AMAP, 1998). Unfortunately, there appears to be no data on effects threshold levels for selenium in marine mammal tissues. Although it has been shown that

marine mammals generally tolerate exceptionally higher threshold concentrations than terrestrial mammals for other metals, such as cadmium and mercury (Eisler, 1985a, 1987; AMAP, 1998).

According to a review of HBV_{Se} in sharks (*Selachimorpha sp.*), pilot whales (*Globicephala sp.*), and large marine fish species, HBV_{Se} results in sea otters of the present study were either at or significantly higher than in species with the highest reported positive HBV_{Se} (yellowfin tuna [*Thunnus albacares*]; Ralston et al., 2015). Selenium is an essential element that is nutritionally important, but harmful at higher concentrations, and the range between beneficial and detrimental levels of selenium is known to be relatively narrow (Eisler, 1985b; AMAP, 1998; USEPA, 2000). For example, dietary selenium levels to maintain human health range between 0.04 and 0.1 ppm, but may become toxic at 4.0 ppm (Eisler, 1985b). Selenium poisoning can cause severe reproductive problems, such as birth defects, growth delay and reduction, respiratory failure, pathological changes to the organs, and death (Eisler, 1985b; AMAP, 1998). Conversely, selenium deficiency causes anemia, slowed growth, and impaired reproduction (Eisler, 1985b). Selenium demonstrates a relationship with several other elements, including arsenic, cadmium, and mercury (Eisler, 1985a, 1985b, 1987, 1988a; Law 1996). It has been shown in several animal species, such as rats (*Rattus sp.*), pigs (*Sus scrofa domesticus*), dogs (*Canis lupus familiaris*), cattle, and fowl (*Galloanserae sp.*), that arsenic can protect against selenium poisoning (Eisler, 1988a). A positive correlation between selenium and cadmium has been demonstrated in the liver tissues of minke whales (*Balaenoptera acutorostrata*), beluga whales (*Delphinapterus leucas*), and narwhals (*Monodon monoceros*) from West Greenland (Law, 1996). Sea otters in the Aleutians have presented a similar relationship (Giger and Trust, 1997).

While the selenium health benefit equation was originally derived as a means of assessing seafood safety criteria by examining the relative effects of mercury exposure and dietary selenium intake for seafood consumption (Ralston et al., 2015), the present study applied this concept as a means of assessing mercury toxicity mitigation by selenium. As it stands, mercury levels in the present study are being positively impacted by corresponding levels of selenium, but one might think those benefits may be diminished if selenium concentrations in these tissues exceeds effects threshold values, in which case,

selenosis is the immediate concern over mercury toxicity. However, it has been shown that high methylmercury concentrations can prevent selenium transport across the placenta and to the brain (Ralston et al., 2015). Selenium and mercury both act to mitigate the other, or counteract each other in keeping either element from reaching highly toxic levels. Evidence also suggests that the relationship between selenium and mercury may not be a 1:1 ratio, but instead that selenium levels may increase as a response to mercury's increase within the tissues (Warburton and Seagars, 1993). Additionally, methylmercury has been shown to make up only 2.7% of the total mercury in livers of ringed seals, 9.0% in narwhals, and ~6-12% in beluga whales (Wagemann et al., 1998). Thus, the most toxic form of mercury (methylmercury) may ultimately make up only a small percentage of the total mercury in body tissues. The present study only analyzed total mercury, but it would be much more effective to analyze both methylmercury and total mercury along with selenium concentrations as a means of assessing overall sea otter health (Wagemann et al., 1998).

2.5. Conclusions and Future Work

The northern sea otter population in Southeast Alaska is quite robust, with population numbers continuing to rise each year (Estes, 1990; Esslinger and Bodkin, 2009; Tinker et al., 2019). Glacier Bay waters tend to be considered "pristine" (Bacon et al., 1999; Carswell et al., 2015), and therefore it was thought that the present study would collect baseline metals concentrations data; that metals concentrations for this study would all be low when compared to studies conducted in other regions and for populations of sea otters that are in decline. However, many of the sea otters in regions compared to the present study had lower metals concentrations than that of the present study (Comerci et al., 2001; Kubota et al., 2001; Kannan et al., 2006, 2008; Brancato et al., 2009). Arsenic concentrations were lower than the present study in other parts of Southeast Alaska, in Southwest Alaska, and in Washington, but were higher in Southcentral Alaska and Baja California. Cadmium concentrations were lower than the present study in all other regions of Alaska, Washington, and Russia, but were higher in California. Copper concentrations were lower than the present study in other parts of Southeast Alaska, Southwest Alaska, and Russia, but equal to or higher in Prince William Sound, Southcentral Alaska, Washington,

and California. Lead concentrations were lower than the present study in Washington, Adak Island, Alaska, Russia, and California, but higher in Prince William Sound. Mercury concentrations were lower than the present study in other parts of Southeast Alaska, Southwest Alaska, and Russia, but higher in Prince William Sound, Washington, and California. Selenium concentrations were lower than the present study in all other regions of Alaska, and Russia, but higher in Washington.

Threshold level effects values are not well documented for marine mammals and those that are available tend to provide large ranges in which adverse effects have been observed (Law, 1996; Ma, 1996). No thresholds have been established specifically for sea otters, which becomes problematic when trying to assess whether the burden of metals contamination found in sea otter tissues is having any deleterious effects on their health or well-being. However, comparing the results of metals concentrations in sea otters' tissues from the present study to thresholds established for other species would indicate that the sea otters of the present study are not experiencing any harmful effects as a direct result of the metals within their tissues. Establishing metals concentrations for sea otters further provides insight into the health of their surrounding local environment (Womble et al., 2018). Since the metals included in this study are naturally occurring (Searight and Moxon, 1945; Eisler, 1985a, 1985b, 1987, 1988, 1998; Law, 1996; Ma, 1996), it was not surprising to find some levels of concentrations in sea otter tissues. However, finding metals concentrations in the present study to be greater in this perceived pristine area (where the sea otter population abounds) as compared to other regions and populations of declining sea otters, was certainly unexpected. The greatest declines in sea otter populations have been witnessed in California though (Kannan et al., 2006, 2008), and that group of sea otters did exhibit much higher metals concentrations than what was found in the present study.

The sea otter population in Icy Strait is currently robust and thriving (Esslinger and Bodkin, 2009; Tinker et al., 2019). Despite this, the findings of the present study demonstrate metals contaminant levels in Icy Strait sea otters are actually higher than metals concentrations in some other regions. This would seem to indicate that the natural mineral deposits of the area and any historic mining efforts in the Glacier Bay National Park and Preserve are not causing adverse effects to the sea otters living there. Therefore, it

is unlikely that these types of contaminants would be the primary cause of sea otter population decline in other ecosystems where these metals have also been measured.

The Southeast Alaska Network of the National Park Service is in the process of implementing a protocol for sea otter monitoring in the Glacier Bay National Park and Preserve, as part of their Vital Signs Monitoring Program (Womble et al., 2018). This program was designed to "monitor the status and trend of key natural resource elements so that park managers can effectively preserve them" (Moynahan and Johnson, 2008). It is obvious that sea otter research is needed, particularly for Glacier Bay and surrounding areas where their population continues to increase annually (Esslinger and Bodkin, 2009; Tinker et al., 2019). Research conducted for the present study could possibly go hand-in-hand with the Glacier Bay National Park and Preserve's Vital Signs Monitoring Program as the present study area was just outside the Park, and perhaps in the future a collaboration with that program could be established.

There is an enormous amount of additional and future work that could follow the research presented within this study. It would be advantageous to continue collaborative research with Alaska Natives to promote stronger ties between researchers and the native community. Alaska Natives typically only utilize the pelt (and possibly some meat) of the sea otters they hunt, and with proper permitting and protocols, researchers can have access to freshly harvested sea otters for any number of research purposes, as was done in the present study and its companion study (Brown et al., 2019). Furthermore, the research conducted for the present study could be continued annually to measure changes in metal concentrations over time. Preferably this research would be continued with a larger sample size which would provide a more robust dataset for this population of sea otters. Measuring metal concentrations in environmental samples such as water and sediment, as well as for sea otter prey items all collected from the sea otter harvest area would provide a much more well-rounded and in depth look at trophic transfer of contaminants from the environment to primary consumer and on up to top level consumers.

Based on anecdotal evidence (M. Gho, personal communication, 2017), it appears the area of the present study may be shifting from primarily male-dominated to more equal numbers of male and female sea otters. If this is the case, future work could include a comparison of metals concentrations between

sexes since male and female mammals may differ in their sequestration and metabolism of contaminants (Warburton and Seagars, 1993; Gochfeld, 2007). Additionally, this study did not evaluate methylmercury separate from total mercury, although methylmercury is considered the most toxic form of mercury. It would make sense for future work to include analyzing both methyl- and total mercury in sea otter tissues along with selenium. While not presented in the present study, laboratory analysis originally included a whole host of organic contaminants in addition to the metals, but the data analysis and presentation of such a vast amount of information was outside the scope of this particular project. In the future, the organics data will be analyzed, and like this study, will be compared with other relevant published literature at that time.

The effects of elevated levels of potentially toxic metals to the health of marine mammals is still largely unknown. The results of the present study represent baseline data since there is limited pre-existing information on contaminant concentrations in healthy sea otter tissues or on sea otters in Southeast Alaska, and none for the area of Glacier Bay (Womble et al., 2018). Glacier Bay waters are typically considered "pristine" (Bacon et al., 1999; Carswell et al., 2015), and it is particularly important to determine levels of contaminants in a pristine environment as a means of comparison for data gathered from less-pristine waters. The results of this study not only add to the Fish Monitoring Program database for monitoring contaminant trends throughout Alaska, but provide a foundation for long-term monitoring of contaminant trends in Southeast Alaska's northern sea otters. Gathering baseline contaminant data in a non-migratory top-level consumer like sea otters, while also knowing the sedentary prey they are feeding upon in this area (Brown et al., 2019) can help assess the health of the surrounding local environment (Bacon et al., 1999; Comerci et al., 2001; Jessup et al., 2004; Kannan et al., 2008; Brancato et al., 2009; Womble et al., 2018).

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2.8. Figures

Figure 2.1. Study area. Map of the northern sea otter (*Enhydra lutris kenyoni*) harvest area in Icy Strait, near Gustavus, Alaska. Fourteen sea otters were harvested in April 2015. Brain, gonad, kidney, and liver tissues, and whole stomachs were collected from each of the sea otters harvested. The stars represent the approximate harvest location for each of the sea otters in the present study; some are overlapping. The map was obtained and modified from the Alaska State Department of Environmental Conservation's Contaminated Sites Program public webmap, available online at: https://www.arcgis.com/home/webmap/viewer.html?webmap=315240bfbaf84aa0b8272ad1cef3cad3.

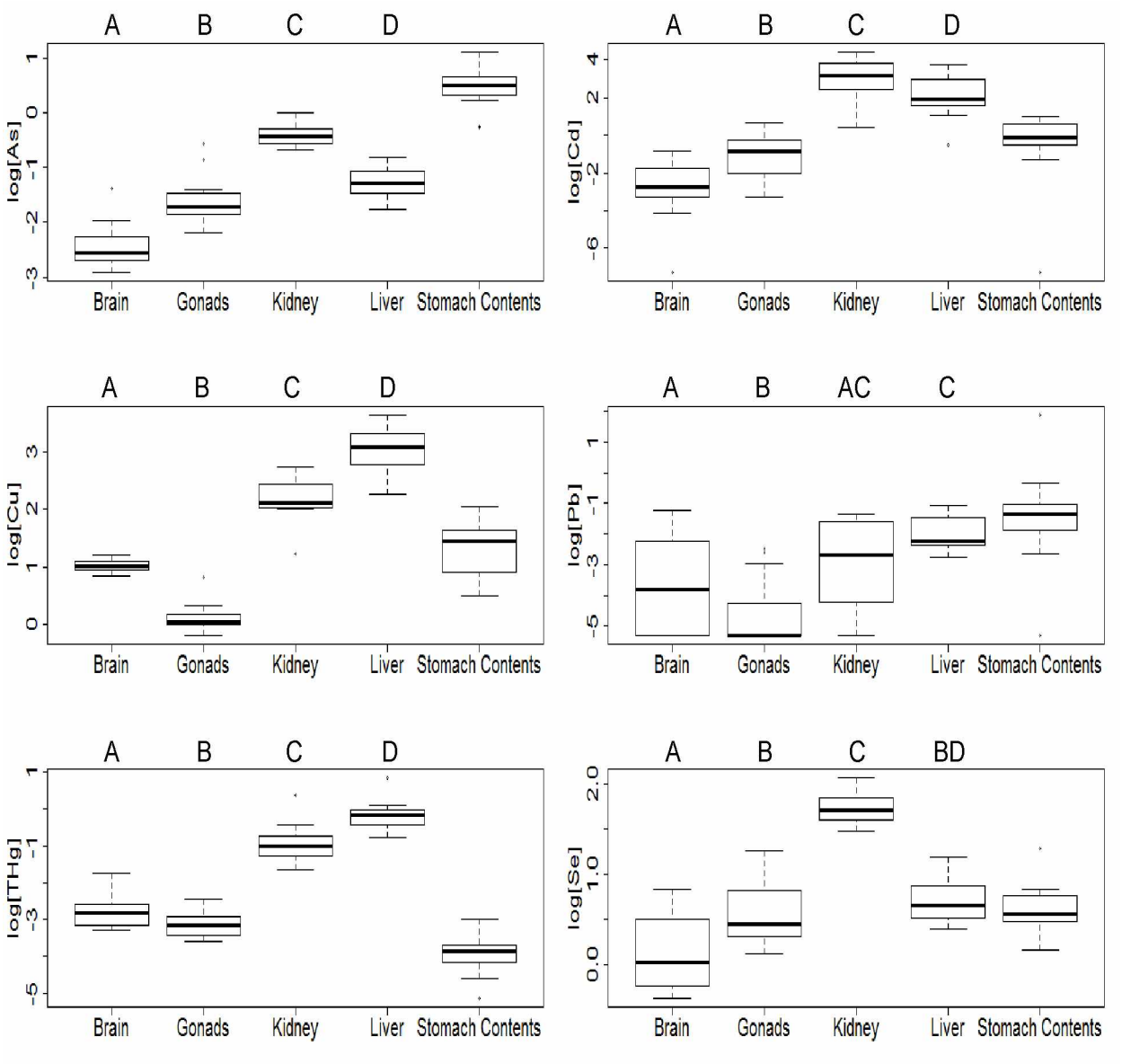

Figure 2.2. Log-transformed metals concentrations by tissue type in sea otters. Boxplots of log-transformed metals concentrations (mg/kg wet weight) for 14 northern sea otters (*Enhydra lutris kenyoni*) by tissue type. A log-transformation of each of the metals concentrations was required to attain approximate normality and equal variances. Metals are abbreviated as follows: As = arsenic, Cd = cadmium, Cu = copper, Pb = lead, THg = total mercury, and Se = selenium. Bold horizontal lines denote median, boxes denote upper and lower quartiles, whiskers denote closest observations falling less than 1.5 times the interquartile range (IQR) outside of the box, and circles denote individual outliers further than 1.5 times the IQR from the box. Letters at the top of each plot denote statistically significant differences in mean concentrations among tissues; those tissues not sharing a common letter are significantly different at $p<0.05$ based on best models in Table 2.4 and Tukey's contrast multiple pairwise comparisons. Stomach contents were considered part of the prey rather than part of the sea otter and excluded from the statistical models; therefore, stomach contents are not included in the pairwise comparisons.

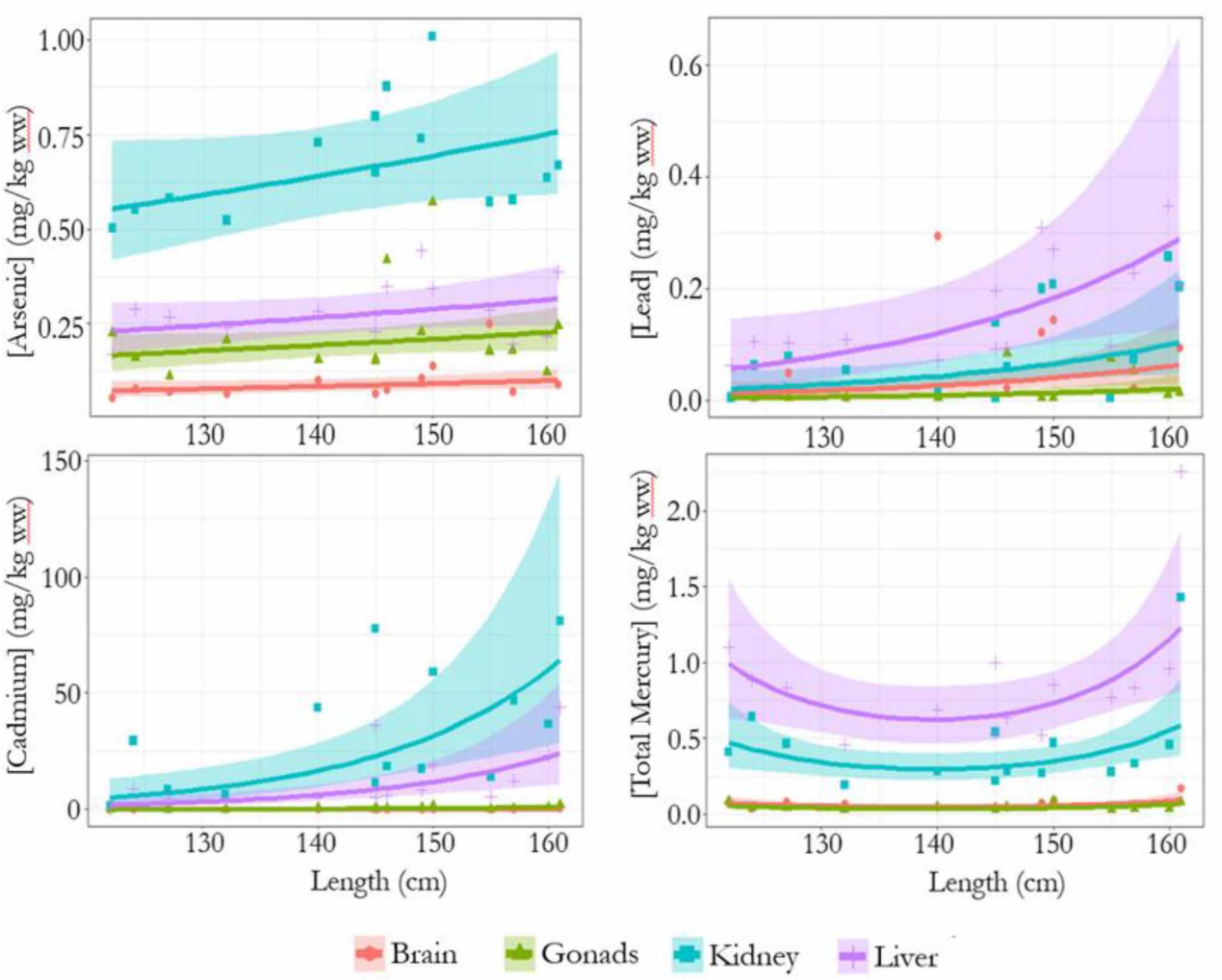

Figure 2.3. Arsenic, cadmium, lead, and total mercury against sea otter length by tissue type. Observed metals concentrations (mg/kg wet weight) plotted against observed length (cm) for 14 northern sea otters (*Enhydra lutris kenyoni*) by brain, gonads, kidney, and liver tissue types. Solid lines denote the fitted values from the best model (Table 2.4) with 95% confidence interval bands.

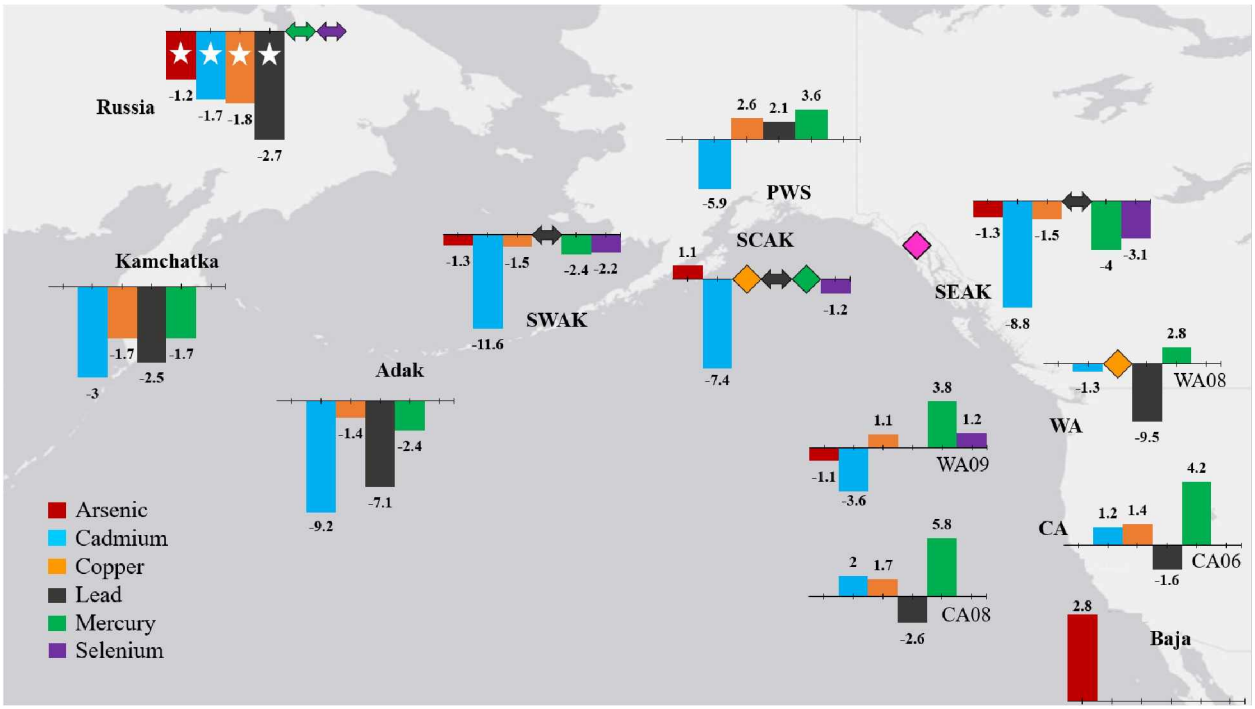

Figure 2.4. Mean metals concentrations in livers of sea otters from the present study as compared to published literature. The present study area acts as the baseline for which the other studies are compared (using dry weight values). The location of the present study is Icy Strait, just outside of Glacier Bay, in Southeast Alaska (represented by the pink diamond). Abbreviated locations are noted as follows: Kamchatka, Russia (Kamchatka), Adak Island, Alaska (Adak), Southwest Alaska (SWAK), Southcentral Alaska (SCAK), Prince William Sound, Alaska (PWS), Southeast Alaska (SEAK), Washington (WA), California (CA), and Baja California (Baja). There are two charts for Washington, one for a study conducted in 2008 (WA08) and one for a study conducted in 2009 (WA09). Similarly, there are two charts for California as well: one for a study conducted in 2006 (CA06) and one for a study conducted in 2008 (CA08). Arsenic is shown in red, cadmium in blue, copper in orange, lead in black, mercury in green, and selenium in purple. Not all charts have all metals represented since already-published studies did not all include every metal shown here. Charts may not be shown exactly to scale. The numbers on each column represent multiples as compared to the present study. For example, mean cadmium concentrations in Kamchatka sea otter livers were 3 times lower than the mean cadmium concentrations in the present study's sea otter livers, and sea otter livers in Baja California had 2.8 times greater mean arsenic concentrations than the sea otter livers of the present study. Stars inside a column indicate a range of values was given in the literature, and the literature's highest end of the range was still multiple times lower than the mean value in the present study. Diamonds in place of a column indicate that the mean concentration of that metal provided in the literature was the same mean concentration found in the present study. Double sided arrows in place of a column indicate that only a range of concentrations was provided in the literature for that metal and the present study's mean value fell within that range. Literature sources and comparison of actual values are detailed in Table 2.7.

2.9. Tables

Table 2.1. Study animals. Northern sea otters (*Enhydra lutris kenyoni*) that were the subjects of the present study with their sex, ages, lengths, weights, and axillary girths (n = 14).

Sea Otter ID	Sex	Age	Length (cm)	Weight (kg)	Axillary Girth (cm)
1	F	10	150	31	58
2	F	1	122	17	51
3	M	8	155	40	66
4	M	2	149	29	61
5	M	4	145	31	64
6	M	3	145	32	69
7	M	2	124	21	61
8	M	1	132	18	51
9	M	2	127	19	52
10	M	4	157	33	62
11	M	7	161	34	61
12	M	5	160	36	69
13	F	10	140	25	58
14	F	2	146	25	53

Table 2.2. Raw data. The raw metals concentrations (mg/kg wet weight) for each sea otter by tissue type. Brain tissue samples from 2 of the 14 sea otters (sea otter IDs #5 and #12) were not collected; sea otter hunters aim for the head when hunting and the two sea otters with missing brain samples did not have enough tissue to collect post-mortem. The stomach collected from sea otter #3 was empty and therefore no contents sample was collected. *The lead data point for the brain tissue of sea otter #3 was removed from analyses due to known contamination of the sample. Results returned as non-detect are marked ND. Italicized values are results that were between the reporting limit (RL) and the method detection limit (MDL); see text for RLs and MDLs of each metal.

Sea Otter ID	Tissue	Arsenic	Cadmium	Copper	Lead	Total Mercury	Selenium
1	Brain	0.139	0.301	3.03	0.143	0.096	2.29
2	Brain	0.0543	ND	3.01	ND	0.072	0.789
3	Brain	0.252	0.0437	2.57	249*	0.046	0.804
4	Brain	0.107	0.182	2.34	0.121	0.074	1.02
5	Brain	NA	NA	NA	NA	NA	NA
6	Brain	0.0665	0.0445	2.67	ND	0.037	0.717
7	Brain	0.0793	*0.016*	2.61	ND	0.037	1.61
8	Brain	0.0648	0.0889	3.32	ND	0.0706	0.696
9	Brain	0.0701	*0.0329*	3.24	ND	0.0792	1.05
10	Brain	0.0704	0.167	2.37	*0.0219*	0.0504	1.71
11	Brain	0.0898	0.431	2.6	0.0937	0.174	1.18
12	Brain	NA	NA	NA	NA	NA	NA
13	Brain	0.1	0.143	2.82	0.294	0.0441	0.834
14	Brain	0.077	*0.0471*	2.95	*0.0212*	0.0418	1.91
1	Gonads	0.571	1.46	2.26	ND	0.087	3.51
2	Gonads	0.226	*0.038*	1	ND	0.086	1.17
3	Gonads	0.178	0.129	0.967	0.074	0.032	1.41
4	Gonads	0.229	0.603	0.828	ND	0.054	3.19
5	Gonads	0.151	0.888	0.989	ND	0.042	1.74
6	Gonads	0.158	0.168	1.02	ND	0.028	1.49
7	Gonads	0.161	0.406	1.38	ND	0.043	1.6
8	Gonads	0.206	0.0906	1.12	ND	0.0278	1.54
9	Gonads	0.111	0.112	1.12	ND	0.0414	1.37
10	Gonads	0.179	0.433	0.879	0.0523	0.0384	2.12
11	Gonads	0.246	1.92	1	*0.014*	0.0824	2.26
12	Gonads	0.121	0.703	1.08	*0.0106*	0.0327	2.37
13	Gonads	0.156	0.755	1.28	ND	0.0488	1.13
14	Gonads	0.42	0.341	1.2	0.0837	0.0508	1.35
1	Kidney	1.01	59.1	7.85	0.208	0.47	7.83
2	Kidney	0.503	1.49	8.92	ND	0.41	4.94
3	Kidney	0.573	14.2	9.69	ND	0.28	5.33
4	Kidney	0.74	17.5	3.43	0.199	0.27	5.49
5	Kidney	0.798	77.8	13	0.139	0.54	6.35
6	Kidney	0.651	11.4	7.53	ND	0.22	4.71
7	Kidney	0.554	29.4	11.4	0.0626	0.642	5.98
8	Kidney	0.524	6.63	8.03	0.054	0.195	5.06
9	Kidney	0.582	8.62	15.7	0.0773	0.463	7.22
10	Kidney	0.578	46.9	7.65	0.0731	0.332	6.29
11	Kidney	0.669	81.1	7.97	0.203	1.43	4.44
12	Kidney	0.637	36.8	8.78	0.257	0.459	5.56
13	Kidney	0.729	43.8	7.38	*0.0149*	0.281	4.4
14	Kidney	0.878	18.5	11.9	0.0586	0.281	6.7

Table 2.2, continued

Sea Otter ID	Tissue	Arsenic	Cadmium	Copper	Lead	Total Mercury	Selenium
1	Liver	0.344	19.3	20.4	0.269	0.85	3.3
2	Liver	0.17	0.585	28.1	0.0619	1.1	1.69
3	Liver	0.287	5.63	22.1	0.098	0.77	1.85
4	Liver	0.444	8.19	9.73	0.309	0.52	2.09
5	Liver	0.272	36.2	35.3	0.196	1	1.91
6	Liver	0.229	4.89	16.1	0.0912	0.53	1.48
7	Liver	0.289	9	23.8	0.104	0.884	1.74
8	Liver	0.239	2.96	14.1	0.108	0.46	1.64
9	Liver	0.268	4.52	15.8	0.103	0.834	2.4
10	Liver	0.197	12.3	16.4	0.227	0.831	2.45
11	Liver	0.387	43.8	27	0.207	2.26	2.18
12	Liver	0.217	23.2	38.3	0.348	0.963	1.97
13	Liver	0.284	4.89	32.7	0.0714	0.689	1.66
14	Liver	0.348	5.91	22.2	0.0938	0.636	2.45
1	Stomach Contents	1.38	0.77	2.26	0.153	0.025	1.5
2	Stomach Contents	0.771	ND	3.2	ND	0.05	1.49
3	Stomach Contents	NA	NA	NA	NA	NA	NA
4	Stomach Contents	3.01	0.264	2.49	0.271	0.01	1.18
5	Stomach Contents	2.04	2.43	4.8	0.36	0.016	2.25
6	Stomach Contents	1.88	0.87	2.8	0.0724	0.011	2.14
7	Stomach Contents	1.26	1.16	5.19	0.226	0.0315	1.75
8	Stomach Contents	1.42	0.838	5.07	0.112	0.0165	1.96
9	Stomach Contents	1.65	1.8	7.14	0.255	0.0212	1.61
10	Stomach Contents	2.56	2.26	6.33	0.601	0.0155	3.59
11	Stomach Contents	1.91	2.69	7.71	0.269	0.0364	1.93
12	Stomach Contents	0.768	1.11	1.66	6.47	0.0211	1.75
13	Stomach Contents	1.68	0.552	1.64	0.17	*0.006*	1.64
14	Stomach Contents	1.65	0.598	4.26	0.722	0.0232	2.29

Table 2.3. Moisture content. The percent moisture content of two sea otter kidneys and one sea otter liver, plus the relative percent difference of the duplicate samples.

Sea Otter ID	Tissue	Moisture Content (%)	Duplicate RPD (%)
4	Kidney	79.32	0.33
12	Kidney	73.35	0.54
12	Liver	71.34	0.78
	Mean ± SD	74.67±4.15	

Table 2.4. Random effects models, ΔAICc, and model weights. Comparison of four random effects models fit via maximum likelihood to quantify variations in sea otter metals concentrations among tissue types and with length. See text for full explanation of models. The bolded set of values under each metal denotes the "best" model chosen for visualizing results.

	Arsenic		Cadmium		Copper		Lead		Total Mercury		Selenium	
Model[a]	ΔAICc	Weight	ΔAICc	Weight	ΔAICc	Weight	ΔAICc	Weight	ΔAICc	Weight	ΔAICc	Weight
1	0.18	0.317	1.96	0.268	4.55	0.070	2.77	0.196	**0.00**	**0.527**	3.18	0.101
2	**0.00**	**0.347**	**0.00**	**0.715**	2.04	0.247	**0.00**	**0.781**	3.02	0.116	0.42	0.402
3	0.06	0.336	7.39	0.018	**0.00**	**0.683**	7.05	0.023	0.78	0.356	**0.00**	**0.496**
4	93.35	0.000	135.12	0.000	148.75	0.000	31.01	0.000	139.78	0.000	98.93	0.000

[a] The models are as follows:

(1) $y_{ik} = \alpha + a_k + \mu_i + \beta_1 L + \beta_2 L^2 + \varepsilon_{ik}$

(2) $y_{ik} = \alpha + a_k + \mu_i + \delta L + \varepsilon_{ik}$

(3) $y_{ik} = \alpha + a_k + \mu_i + \varepsilon_{ik}$

(4) $y_{ik} = \alpha + a_k + \varepsilon_{ik}$

Table 2.5. Main model effects ($\mu_i, \beta_1, \beta_2, \delta$) ± standard errors (SE), the standard deviation of the random intercept (σ_ε^2), and the residual standard deviation (ε_{ik}) for each metal. The main model effects for each of the metals using their respective "best" models fit via restricted maximum likelihood (REML). See text for full explanation of models. See Table 2.4 for each metals' chosen best model.

	Arsenic	Cadmium	Copper	Lead	Total Mercury	Selenium
μ_{brain}	-3.58±0.72	-11.95±2.62	1.04±0.09	-9.51±1.74	-2.78±0.11	0.12±0.08
μ_{gonads}	-2.76±0.72	-10.32±2.63	0.11±0.08	-10.63±1.76	-3.08±0.11	0.57±0.08
μ_{kidney}	-1.56±0.72	-6.22±2.63	2.17±0.08	-9.02±1.76	-0.95±0.11	1.73±0.08
μ_{liver}	-2.44±0.72	-7.20±2.63	3.07±0.08	-7.99±1.76	-0.21±0.11	0.70±0.08
β_1	NA	NA	NA	NA	0.41±0.64	NA
β_2	NA	NA	NA	NA	1.50±0.63	NA
δ	0.01±0.01	0.06±0.02	NA	0.04±0.01	NA	NA
σ_ε^2	0.19	0.81	0.16	0.14	0.29	0.22
ε_{ik}	0.28	0.54	0.26	1.08	0.27	0.21

Table 2.6. Selenium health benefit values (HBV_{Se}) and biomagnification factors (BMFs). Means ± standard deviations of HBV_{Se} (Ralston et al., 2015) and the BMFs (adapted from Cardoso et al., 2014; originally from Hoekstra et al., 2003) of metals in the brain, gonad, kidney, and liver tissues as compared to the stomach contents of 14 northern sea otters (*Enhydra lutris kenyoni*) from Icy Strait, Alaska. See text for formulas and explanations.

		BMFs					
Tissue Type (T)	HBV_{Se}	Arsenic	Cadmium	Copper	Lead	Total Mercury	Selenium
Brain	15.41±6.46	0.06	0.11	0.67	0.09	3.14	0.63
Gonads	23.74±9.30	0.13	0.49	0.27	0.03	2.27	0.97
Kidney	72.53±13.28	0.40	27.43	2.20	0.13	20.55	2.97
Liver	25.15±6.15	0.17	10.98	5.48	0.22	40.39	1.07
Stomach Contents	24.43±7.51	NA	NA	NA	NA	NA	NA

Table 2.7. Literature summary. Summary of mean metals concentrations (± standard deviations, where applicable) in sea otters (*Enhydra lutris*) from relevant literature sources. Results are reported as mg/kg wet weight, with corresponding mg/kg dry weight concentrations in italics. Ranges are listed in parentheses (both mg/kg wet weight in regular font and mg/kg dry weight in italics) if they were provided in the literature. Studies were conducted in Icy Strait, Alaska, Southeast Alaska (SEAK), Southcentral Alaska (SCAK), Southwest Alaska (SWAK), Prince William Sound, Alaska (PWS), Adak Island, Alaska, Russia (RUS), Washington (WA), California (CA), Baja California, or some combination of regions. Sample sources were freshly harvested via subsistence hunting (S), beach-cast carcasses (B), or both (S & B).

Region	Sample Source	N	Tissue Type	Arsenic	Cadmium	Copper	Lead	Mercury	Selenium	Source/ Study
Icy Strait, AK	S	14	Kidney	0.67±0.14 (0.50-1.01) *2.85±0.61* *(2.13-4.27)*	32.37±26.15 (1.49-81.10) *136.93±110.60* *(6.30-343.05)*	9.23±2.98 (3.43-15.70) *39.05±12.62* *(14.51-66.41)*	0.10±0.09 (ND-0.26) *0.41±0.37* *(0.02-1.09)*	0.45±0.31 (0.20-1.43) *1.90±1.32* *(0.82-6.05)*	5.74±1.04 (4.40-7.83) *24.26±4.41* *(18.61-33.12)*	Present Study
SEAK	S & B	21	Kidney	-- *1.75* *(<0.5-4.49)*	-- *19.62* *(2.67-69.18)*	-- *19.01* *(11.4-42.8)*	-- -- *(<0.5-0.98)*	-- *0.983* *(<0.1-2.23)*	-- *5.21* *(2.56-11.0)*	Comerci et al., 2001
SCAK	S & B	22	Kidney	-- *2.16* *(<0.5-6.06)*	-- *20.58* *(1.44-214)*	-- *18.47* *(10.2-29.8)*	-- -- *(<0.5-0.53)*	-- *1.09* *(<0.2-10.7)*	-- *8.31* *(1.56-22.9)*	Comerci et al., 2001
SWAK	S & B	16	Kidney	-- *1.79* *(<0.5-3.93)*	-- *14.17* *(<0.1-179)*	-- *15.46* *(7.7-25.2)*	-- --	-- *0.063* *(<0.1-4.66)*	-- *5.52* *(0.89-22.7)*	Comerci et al., 2001
RUS	S & B	2	Kidney	-- -- *(0.82-2.17)*	-- -- *(14.9-87.9)*	-- -- *(18.4-23.7)*	-- --	-- -- *(0.43-0.61)*	-- -- *(7.19-7.36)*	Comerci et al., 2001
SEAK, SCAK, SWAK, and RUS **Combined**	S & B	61	Kidney	0.53 *1.88* *(<0.5-6.06)*	5.24 *18.70* *(<0.1-214)*	-- *17.87* *(7.7-42.8)*	-- (ND-0.30) -- *(<0.5-0.99)*	0.19 *0.70* *(<0.1-10.7)*	1.77 *6.33* *(0.89-22.9)*	Comerci et al., 2001
Icy Strait, AK	S	14	Liver	0.28±0.08 (0.17-0.44) *0.99±0.26* *(0.59-1.55)*	12.96±13.11 (0.59-43.80) *45.21±45.74* *(2.04-152.9)*	23.00±8.45 (9.73-38.30) *80.28±29.50* *(33.96-133.67)*	0.16±1.73 (0.06-0.35) *0.57±0.33* *(0.22-1.21)*	0.88±0.44 (0.46-2.26) *3.07±1.54* *(1.61-7.89)*	2.06±0.48 (1.48-3.30) *7.18±1.67* *(5.17-11.52)*	Present Study

Table 2.7, continued

Region	Sample Source	N	Tissue Type	Arsenic	Cadmium	Copper	Lead	Mercury	Selenium	Source/ Study
SEAK	S & B	21	Liver	-- *0.753* *(<0.5-1.47)*	-- *5.15* *(1.68-24.78)*	-- *52.98* *(28.0-87.8)*	-- -- *(<0.5-0.64)*	-- *0.771* *(<0.1-1.74)*	-- *2.33* *(1.1-6.21)*	Comerci et al., 2001
SCAK	S & B	29	Liver	-- *1.042* *(<0.5-3.38)*	-- *6.09* *(0.55-31.0)*	-- *84.02* *(33.4-227.0)*	-- -- *(<0.5-1.67)*	-- *3.04* *(0.04-15.7)*	-- *6.07* *(1.59-16.7)*	Comerci et al., 2001
PWS, AK	B	2	Liver	-- --	-- *7.6* *(6.4-8.9)*	-- *210* *(99-320)*	-- *1.2* *(0.93-1.4)*	-- *11* *(10-11)*	-- --	Kannan et al., 2008
WA	B	15	Liver	0.27±0.11 *0.92±0.49*	3.7±4.0 *12.6±14.2*	25.9±16.2 *86.5±53.1*	-- --	3.4±3.4 *11.6±10.8*	2.54±1.58 *8.6±5.2*	Brancato et al., 2009
WA[a]	B	3	Liver	-- --	-- *35±29*	-- *84±37*	-- *0.06±0.02*	-- *8.6±8.1*	-- --	Kannan et al., 2008
Adak Island, AK	B	2	Liver	-- --	-- *4.9* *(3.6-6.2)*	-- *56* *(25-87)*	-- *0.08* *(0.03-0.12)*	-- *1.3* *(0.74-1.9)*	-- --	Kannan et al., 2008
SWAK	S & B	16	Liver	-- *0.74* *(<0.5-1.98)*	-- *3.90* *(<0.1-20.1)*	-- *54.24* *(4.29-175)*	-- -- *(<0.5-0.95)*	-- *1.26* *(0.23-12.8)*	-- *3.29* *(1.18-16.6)*	Comerci et al., 2001
SEAK, SCAK, SWAK, and RUS **Combined**	S & B	68	Liver	0.25 *0.88* *(<0.5-3.38)*	1.48 *5.31* *(<0.1-31.0)*	-- *64.05* *(4.29-227)*	0.52 -- *(<0.5-1.66)*	0.43 *1.55* *(<0.1-15.7)*	1.10 *3.93* *(1.1-16.7)*	Comerci et al., 2001
RUS	S & B	2	Liver	-- -- *(0.62-0.84)*	-- -- *(4.98-26.6)*	-- -- *(26.6-45.2)*	-- --	-- -- *(0.49-1.12)*	-- -- *(6.23-7.99)*	Comerci et al., 2001
Kamchatka, RUS	B	5	Liver	-- --	-- *15±12*	-- *47±13*	-- *0.23±0.11*	-- *1.8±1.8*	-- --	Kannan et al., 2008

Table 2.7, continued

Region	Sample Source	N	Tissue Type	Arsenic	Cadmium	Copper	Lead	Mercury	Selenium	Source/ Study
CA	B	6	Liver	-- --	-- *53±70*	-- *110±42*	-- *0.35±0.39*	-- *13±6.6*	-- --	Kannan et al., 2008
CA	B	80	Liver	-- --	-- *91.9* *(<0.01-728)*	-- *133* *(26.3-401)*	-- *0.22* *(0.02-1.1)*	-- *17.8* *(0.48-128)*	-- --	Kannan et al., 2006
Baja California	S & B	10	Liver	-- *2.73±1.78* *(0.77-5.70)*	-- --	-- --	-- --	-- --	-- --	Kubota et al., 2001
Icy Strait, AK	S	12	Brain	0.10±0.05 (0.05-0.25) *0.39±0.22*	0.12±0.13 (ND-0.43) *0.50±0.52*	2.79±0.32 (2.34-3.32) *11.18±1.28*	0.07±0.09[b] (ND-0.29) *0.25±0.35*	0.07±0.04 (0.04-0.17) *0.27±0.15*	1.22±0.53 (0.70-2.29) *4.87±2.13*	Present Study
Icy Strait, AK	S	14	Gonads	0.22±0.13 (0.11-0.57) *0.89±0.50*	0.57±0.55 (0.04-1.92) *2.29±2.20*	1.15±0.35 (0.83-2.26) *4.61±1.41*	0.02±0.03 (ND-0.08) *0.08±0.11*	0.05±0.02 (0.03-0.09) *0.20±0.08*	1.88±0.73 (1.13-3.51) *7.50±2.94*	Present Study
Icy Strait, AK	S	13	Stomach Contents	1.69±0.63 (0.77-3.01) *6.76±2.52*	1.18±0.85 (ND-2.69) *4.72±3.41*	4.20±2.05 (1.64-7.71) *16.78±8.19*	0.75±1.73 (ND-6.47) *2.98±6.93*	0.02±0.01 (0.01-0.05) *0.09±0.05*	1.93±0.59 (1.18-3.59) *7.72±2.37*	Present Study

[a] Washington sea otters used in the Kannan et al., 2008 study likely include some of the sea otters used in the Brancato et al., 2009 study.

[b] The lead data point for the brain tissue of one animal was removed due to known contamination of the sample; therefore, the mean, standard deviation, and range for lead in brain tissue was calculated using $n = 11$.

Table 2.8. Means ± standard deviations, and range of metal concentrations for sea otters by tissue type. Concentrations are displayed in mg/kg wet weight with the calculated mg/kg dry weight in italics. Dry weights were calculated using 76.34% moisture content for kidneys, 71.34% moisture content for livers, and 75.00% moisture content for all other tissues (see text for equations and further explanation). Concentrations of metals that were returned as non-detect (ND) were evaluated at half their method detection limit.

Tissue Type		Arsenic	Cadmium	Copper	Lead	Total Mercury	Selenium
Brain (n=12)	ww	0.10±0.05	0.12±0.13	2.79±0.32	*0.07±0.09	0.07±0.04	1.22±0.53
	dw	*0.39±0.22*	*0.50±0.52*	*11.18±1.28*	*0.25±0.35*	*0.27±0.15*	*4.87±2.13*
Gonads (n=14)	ww	0.22±0.13	0.57±0.55	1.15±0.35	0.02±0.03	0.05±0.02	1.88±0.73
	dw	*0.89±0.50*	*2.29±2.20*	*4.61±1.41*	*0.08±0.11*	*0.20±0.08*	*7.50±2.94*
Kidney (n=14)	ww	0.67±0.14	32.37±26.15	9.23±2.98	0.10±0.09	0.45±0.31	5.74±1.04
	dw	*2.85±0.61*	*136.94±110.60*	*39.05±12.62*	*0.41±0.37*	*1.90±1.32*	*24.26±4.41*
Liver (n=14)	ww	0.28±0.08	12.96±13.11	23.00±8.45	0.16±1.73	0.88±0.44	2.06±0.48
	dw	*0.99±0.26*	*45.21±45.74*	*80.28±29.50*	*0.57±0.33*	*3.07±1.54*	*7.18±1.67*
Stomach Contents (n=13)	ww	1.69±0.63	1.18±0.85	4.20±2.05	0.75±1.73	0.02±0.01	1.93±0.59
	dw	*6.76±2.52*	*4.72±3.41*	*16.78±8.19*	*2.98±6.93*	*0.09±0.05*	*7.72±2.37*
Range	ww	0.05–3.01	0.00–81.10	0.83–38.30	0.01–6.47	0.01–2.26	0.70–7.83
	dw	*0.20–12.04*	*0.00–324.40*	*3.32–153.20*	*0.04–25.88*	*0.04–9.04*	*2.8–31.32*

* The lead data point for the brain tissue of one animal was removed due to known contamination of the sample; therefore, the mean and standard deviation for lead in brain tissue was calculated using $n = 11$.

General Conclusions

Sea otters (*Enhydra lutris*) are keystone species capable of significantly restructuring their surrounding environment (Estes and Palmisano, 1974; Garshelis et al., 1986; Estes, 1990; Jessup et al., 2004). Sea otters are an ideal species for studying their local ecosystem health as they are relatively sedentary, remain within small home ranges near the coastline (they do not migrate), and they are top trophic level consumers (Bacon et al., 1999; Comerci et al., 2001; Jessup et al., 2004; Kannan et al., 2008; Brancato et al., 2009). They target sedentary prey which filter feed on sediments and detritus (or kelp [Order Laminariales] in the case of sea urchins [*Echinoidea sp.*]), and can ingest and concentrate a variety of environmental contaminants (Bacon et al., 1999; Jessup et al., 2004; Carswell et al., 2015).

Chapter 1 examined sea otter diet composition through analysis of stomach contents. While sea urchins are considered an important component of sea otter diet in rocky habitats (Watt et al., 2000; Estes et al., 2003; Laidre and Jameson, 2006; Newsome et al., 2015), no sea urchins were discovered in any of the sea otter stomachs collected from the soft-sediment habitat of Icy Strait. Instead, the diet is primarily dominated by bivalves. Northern horsemussels (*Modiolus modiolus*) make up the greatest proportion of sea otters' diet in this area (0.46 ± 0.48). Fat gaper clams (*Tresus capax*) and northern horsemussels were found in the highest proportion of sea otter stomachs (0.64 and 0.60, respectively). There were no apparent trends indicated between the age of a sea otter with both the minimum number of total prey inside its stomach and the mass of its stomach contents. This points to the possibility that sea otters of all ages might be eating the same amount of prey items and if so, that young sea otter stomachs could potentially hold the same mass of prey that older sea otter stomachs hold.

There are two main reasonings that might explain why bivalves are dominating sea otters' diet in Icy Strait. (1) Sea otters inhabiting Icy Strait are what is considered an established population (>25 years of occupancy), and as sea otters deplete preferred prey sources post initial colonization of an area, their prey preference shifts to a less specialized, but more abundant bivalve-dominated diet (Kvitek et al., 1993; Laidre and Jameson, 2006). (2) Sea otters from areas of mixed- or soft-sediment benthos (such as that of the present study area) generally tend to have diets that are dominated by infaunal bivalves

(Garshelis et al., 1986; Riedman and Estes, 1990; Kvitek et al., 1993). Icy Strait is a soft-sediment benthos and therefore the predominance of bivalves found in sea otter diets from this area is likely a reflection of that soft substrate type from which they were collected. This study and others conducted in soft- and mixed- sediment habitats have concluded sea otters to be dietary generalists (Kvitek and Oliver, 1992; Wolt et al., 2012).

This research presented data on sea otter prey to help illustrate potential dietary exposure to metals contamination. Chapter 2 evaluated metals concentrations in four different sea otter tissues (brain, gonads, kidney, and liver) as well as metals concentrations of their prey (i.e., the stomach contents). While I found that some of the metals have relationships with length (arsenic, cadmium, lead, and mercury), the greatest amount of variability for all metals is actually attributed to the tissue itself. In general, arsenic and lead have the highest concentrations in stomach contents, cadmium and selenium are highest in the kidneys, and copper and total mercury are highest in the livers. Cadmium, copper, and selenium biomagnified in kidney and liver tissues, but only total mercury demonstrated biomagnification from the stomach contents to all higher-level tissues. Generally, brain tissue and gonads had low concentrations of all metals.

The northern sea otter (*E. l. kenyoni*) population in Southeast Alaska is quite robust, as population numbers continue to rise each year. Because Glacier Bay waters tend to be considered pristine, it was expected that all of the metals concentrations for this study would all be low (especially when compared to studies conducted in other regions or for populations of sea otters that are in decline). However, many of the regions I compared my results to had lower metals concentrations than that of my study (Comerci et al., 2001; Kubota et al., 2001; Kannan et al., 2006, 2008; Brancato et al., 2009). Arsenic concentrations were lower than my study in other parts of Southeast Alaska, in Southwest Alaska, and in Washington, but were higher in Southcentral Alaska and Baja California. Cadmium concentrations were lower than my study in all other regions of Alaska, Washington, and Russia, but were higher in California. Copper concentrations were lower than my study in other parts of Southeast Alaska, Southwest Alaska, and Russia, but equal to or higher in Prince William Sound, Southcentral Alaska, Washington, and California.

Lead concentrations were lower than my study in Washington, Adak Island, Alaska, Russia, and California, but higher in Prince William Sound. Mercury concentrations were lower than my study in other parts of Southeast Alaska, Southwest Alaska, and Russia, but higher in Prince William Sound, Washington, and California. Selenium concentrations were lower than my study in all other regions of Alaska, and Russia, but higher in Washington.

Threshold level effects values are not well documented for marine mammals and those that are available tend to provide large ranges in which adverse effects have been observed (Law, 1996; Ma, 1996). No thresholds have been established specifically for sea otters, which becomes a problem when trying to assess whether the burden of metals contamination found in sea otter tissues is having any deleterious effects on their health or well-being. However, establishing metals concentrations for sea otters still provides insight into the health of their surrounding local environment. Since all of the metals included in this study are naturally occurring (Searight and Moxon, 1945; Eisler, 1985a, 1985b, 1987, 1988, 1998; Law, 1996; Ma, 1996), it is not a surprise to find some levels of concentrations in sea otter tissues. However, it is a bit surprising to find these concentrations are greater in a perceived pristine area where the sea otter population abounds (as compared to other regions/populations of sea otters). However, the greatest declines in sea otter populations have been witnessed in California (Kannan et al., 2006, 2008), which did exhibit much higher metals concentrations than what was found in my study.

My findings show that although metals contaminant levels in Icy Strait sea otters that live just outside of Glacier Bay National Park and Preserve are actually higher than metals in some other regions, the sea otters in Icy Strait are doing exceptionally well. This seems to indicate that the natural mineral deposits of the area and any historic mining efforts in the Glacier Bay National Park and Preserve area are not causing any adverse effects to the sea otters living there. Therefore, it is unlikely that these types of contaminants would be the primary cause of sea otter population decline in other ecosystems where these metals have also been measured.

This study is unique in that all of the samples were collected fresh in collaboration with Alaska Native subsistence hunters. Research such as this promotes stronger ties between researchers and the

native community since this study would not have been possible without Alaska Natives and in turn, researchers can provide health and status updates on the animals upon which Alaska Natives subsist. Alaska Natives are deeply invested in their subsistence resources and many wish to be included in helping assess the health of the areas they hunt within. Healthy ecosystems mean that Alaska Natives can continue harvesting from these areas for years to come.

More and more, sea otters are being recognized for the role they play in determining ecosystem health. Glacier Bay National Park and Preserve is implementing a sea otter monitoring protocol as part of the Vital Signs Monitoring Program designed to "monitor the status and trend of key natural resource elements so that park managers can effectively preserve them" (Moynahan and Johnson, 2008). It is obvious that sea otter research is needed, particularly for Glacier Bay where their population continues to increase annually, subsequently causing concern regarding their effects to commercial fisheries, and indicating the necessity for strategic management of both sea otters and commercially important fishery resources. The results of studies on dietary exposure and metals contamination in top trophic level consumers such as sea otters can be used in monitoring the health of sea otter populations and the local environment that they inhabit.

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DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE

FEDERAL FISH AND WILDLIFE PERMIT

2. AUTHORITY-STATUTES
16 USC 1371 (a) (1)

REGULATIONS
50 CFR 18.31

1. PERMITTEE

SHANNON K. ATKINSON PHD
SCHOOL OF FISHERIES AND OCEAN SCIENCE
UNIVERSITY OF ALASKA FAIRBANKS, JUNEAU CTR
17101 PT. LENA LOOP ROAD
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U.S.A.

3. NUMBER
MA81899A-0

4. RENEWABLE ☒ YES ☐ NO

5. MAY COPY ☒ YES ☐ NO

6. EFFECTIVE
02/11/2013

7. EXPIRES
02/10/2018

8. NAME AND TITLE OF PRINCIPAL OFFICER *(If it is a business)*

9. TYPE OF PERMIT
MARINE MAMMAL SCIENTIFIC RESEARCH

10. LOCATION WHERE AUTHORIZED ACTIVITY MAY BE CONDUCTED

11. CONDITIONS AND AUTHORIZATIONS:

A. GENERAL CONDITIONS SET OUT IN SUBPART D OF 50 CFR 13, AND SPECIFIC CONDITIONS CONTAINED IN FEDERAL REGULATIONS CITED IN BLOCK #2 ABOVE, ARE HEREBY MADE A PART OF THIS PERMIT. ALL ACTIVITIES AUTHORIZED HEREIN MUST BE CARRIED OUT IN ACCORD WITH AND FOR THE PURPOSES DESCRIBED IN THE APPLICATION SUBMITTED. CONTINUED VALIDITY, OR RENEWAL, OF THIS PERMIT IS SUBJECT TO COMPLETE AND TIMELY COMPLIANCE WITH ALL APPLICABLE CONDITIONS, INCLUDING THE FILING OF ALL REQUIRED INFORMATION AND REPORTS.

B. THE VALIDITY OF THIS PERMIT IS ALSO CONDITIONED UPON STRICT OBSERVANCE OF ALL APPLICABLE FOREIGN, STATE, LOCAL, TRIBAL, OR OTHER FEDERAL LAW.

C. VALID FOR USE BY PERMITTEE NAMED ABOVE.

D. Acceptance of this permit serves as evidence that the permittee understands and agrees to abide by the "General Permit Conditions" (copy attached).

E. For the purpose of scientific research on the reproductive strategies and the dynamics of the population of animals in southeast Alaska, Permittee is authorized to acquire samples from up to 125 carcasses of northern sea otters (Enhydra lutris kenyoni) annually, of either sex and any age class, removed from the wild by for subsistence purposes by Alaska native hunters in the waters of southeast Alaska as described in Permittee's application and as conditioned below. Samples include skinned carcass, reproductive tract, a pre-molar tooth, and a blood sample.

F. Only animals from southeast Alaska may be obtained; for each carcass, the exact location of where the animal was hunted must be provided (see Condition J.1) below).

CONDITIONS CONTINUE ON PAGE 2

☒ ADDITIONAL CONDITIONS AND AUTHORIZATIONS ALSO APPLY

12. REPORTING REQUIREMENTS
SUBMIT COMPLETE REPORT AS REQUIRED BY CONDITION J. TO: DMA AND MMM BY 1/31 FOLLOWING EACH YEAR PERMIT IS IN EFFECT.

ISSUED BY

TITLE FOR
CHIEF, BRANCH OF PERMITS, DMA

DATE
02/11/2013

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Washington, D.C. 20240

G. **Shannon K. Atkinson, Ph.D, is hereby designated as principle investigator (PI) under this permit, and Emily Hutchinson is designated as Co-investigators (CI).** The PI may designate any other personnel as Co-investigator(s), provided the individuals have received appropriate training and possess adequate proficiency to conduct the research activities in accordance with the permit conditions. Upon designation of additional Co-investigator(s), the Permittee must submit the individuals' CVs to the Division of Management Authority (DMA).

H. **Permittee and all authorized personnel (see Condition G) must have a copy of this permit and, if applicable, all other written approvals in possession while conducting all authorized activities.**
1) Activities under this permit must be conducted in accordance with U.S.G.S. Institutional Animal Care and Use Committee standards and under the on-site supervision of the PI or a CI.
2) All written approvals must remain on file at a designated repository at permittee institution for a period of 5 years. A list of the approved personnel must be submitted to DMA upon request.

I. Permittee may transfer teeth samples to Matson's Laboratory, Montana.

J. Annual Reporting Requirements. In accordance with block 12 of this permit, copies of an Annual Report of the previous year's activities must be submitted by January 31 to DMA: 4401 North Fairfax Drive, Room 212, Arlington, Virginia 22203 and to MMM, 1011 East Tudor Road, Anchorage, AK 99503. The Annual Report shall include at a minimum the following:

1) Tabulation of sea otters acquired indicating: age class, sex, weight, tissue samples taken, location of hunt, and date animals removed from the wild.
2) Discussion of any problems or complications encountered during the research.
3) Discussion of study results, including how this research complements previous research.
4) List of approved personnel.
5) The final report should include a summary of data analyses, results, conclusions, and copies of any published research findings.

K. If Permittee desires to change study procedures from that previously described in the Permittee's file, then a letter must be submitted to DMA describing the proposed changes, and confirmation that the proposed changes fall within the authorized takes in the permit must be received from DMA prior to undertaking the procedural modifications.

L. The authorized permit activities may be extended beyond the expiration date **only** if the renewal request is received by the DMA **at least 30 days prior to the expiration of the permit** [50 CFR 13.22(c); copy attached].

FEB 11 2013
DATE

for Chief, Branch of Permits
Division of Management Authority